

Selecting rootstocks to improve vine performance and vineyard sustainability in deficit irrigated Monastrell grapevines under semiarid conditions

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ABSTRACT

Regulated deficit irrigation (RDI) and partial root zone irrigation (PRI) were compared for five years, in field-grown mature Monastrell grapevines grafted on five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C), in the semiarid winegrowing region of D.O. Bullas, South Eastern Spain. Vines grafted on invigorating rootstocks (140Ru or 1103 P) showed the highest vigor, water productivity, and productive water use efficiency (WUE_{yield}), but at the expense of berry quality (lower berry quality indices, QI), compared to those on rootstocks of medium-low vigor (41B, 110R, and 161-49C). Vines grafted on 41B showed a moderate vigor-yield-efficiency-quality response, and this did not improve substantially the final berry quality. The least vigorous rootstocks (161-49C and 110R) gave lower yield, WUE_{yield} , and productivity ratios, but a significant improvement in long-term final berry quality. The PRI increased the yield and/or berry quality attributes, especially in low vigor rootstocks (161-49C, 110R) and high vigor rootstocks (140Ru, 1103 P), but not in the medium vigor rootstock 41B. In addition, PRI produced a beneficial increase in the nutraceutical potential for practically all rootstocks. The PRI vines grafted on 161-49C gave the lowest yield and WUE_{yield} , but the highest QI scores and the highest nutraceutical value, while PRI vines grafted on 110R had enhanced long-term yield, WUE_{yield} , and amino acid and resveratrol contents, with similar berry quality (QI) indices, compared to RDI vines. Both 161-49C and 110R seem good options to achieve a compromise between long-term yield-quality-efficiency and returns for the grower. The application of low water volumes ($85\text{--}90\text{ mm year}^{-1}$) with well-designed DI strategies was enough to maintain the vines in an optimum physiological state, obtaining moderate yields ($7,400\text{--}9,900\text{ kg ha}^{-1}$, for 161-49C and 110R) with high berry quality and nutraceutical potential for premium red wine production. Such an approach can serve as an adaptation measure in the face of climate change, to improve vine performance and enhance Monastrell vineyard sustainability under semiarid and water limiting conditions.

1. Introduction

According to the climate projections for the middle of this century (2040–2070), temperature, aridity, and water stress are expected to increase in many areas of southern Europe, reducing yield and growth of the vine (Fraga et al., 2013, 2016; IPCC, 2014; Resco, 2015; Guiot and Cramer, 2016). In addition, the increase in extreme temperatures may lead to an increased risk of pests and diseases (Fraga et al., 2013) and a decrease in quality and yield due to recurrent heat shock, especially in the more continental and warmer areas from the center and south of the Iberian Peninsula. It would be in these semiarid regions of southern Europe (e.g. SE Spain) where viticulture would need to make the greatest efforts of adaptation, with greater costs to maintain quality

and productivity, since they are going to experience more severe water stress and impacts of greater magnitude than other wine-growing areas (Resco et al., 2016). In these warm and more vulnerable regions it will be necessary to combine different adaptation measures, especially those related to water management and availability (Iglesias and Garrote, 2015).

Water scarcity is one of the most important problems of agriculture in semiarid regions. Increased aridity and reduced rainfall (predicted in the future) will increase water scarcity, making the availability of water for irrigation an even more limiting factor for agriculture. The predicted increase in evapotranspiration and in the water needs of the vine as a result of climate change (CC) will make necessary the application of irrigation water to maintain the sustainability of vineyards and to

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Table 1

Mean values of several climatic parameters in different phenological periods for every year of the experiment in the study area.

Phenological period	Period of the year	ETo (mm)	VPD (kPa)	Rainfall (mm)	T ^o max (°C)	T ^o med. (°C)	T ^o min (°C)	Solar rad. (Wm ⁻²)
Year 2012								
Budburst-fruit set	1 April–1 June	281	1.08	30	23.3	16.0	7.3	270
Fruit set-veraison	2 June–31 July	379	1.90	2	32.3	24.3	14.5	321
Veraison-harvest	1 August–24 Sept	272	1.84	9	32.7	24.0	14.3	252
Postharvest	25 Sept–31 Octob.	78	0.61	185	22.4	15.4	9.2	155
Dormancy period	1 Nov–31 March	242	0.39	169	15.2	7.5	0.8	130
Total/annual average		1,253	1.00	395	22.7	14.9	6.9	209
Year 2013								
Budburst-fruit set	1 April–1 June	241	0.80	78	21.4	14.5	7.0	249
Fruit set-veraison	2 June–31 July	355	1.70	4	30.4	22.2	11.6	320
Veraison-harvest	1 August–26 Sept	257	1.29	39	30.3	22.4	14.3	247
Postharvest	27 Sept–31 Octob.	91	0.94	4	26.3	18.2	10.7	164
Dormancy period	1 Nov–31 March	267	0.50	119	15.4	8.1	1.1	128
Total/annual average		1,211	0.95	244	22.3	14.7	6.9	206
Year 2014								
Budburst-fruit set	1 April–1 June	298	1.19	28	25.0	16.9	7.6	274
Fruit set-veraison	2 June–31 July	361	1.75	8	31.3	23.4	14.3	307
Veraison-harvest	1 August–18 Sept	264	1.80	0	32.9	24.6	15.2	272
Postharvest	19 Sept–31 Octob.	111	0.91	20	25.7	17.9	10.6	162
Dormancy period	1 Nov–31 March	276	0.51	77	16.4	9.0	2.1	127
Total/annual average		1,309	1.06	133	23.6	15.9	7.8	207
Year 2015								
Budburst-fruit set	1 April–1 June	266	1.09	24	24.3	16.6	8.2	260
Fruit set-veraison	2 June–31 July	373	2.02	16	33.3	24.6	14.4	312
Veraison-harvest	1 August–18 Sept	227	1.45	79	30.9	23.7	16.5	238
Postharvest	19 Sept–31 Octob.	102	0.73	50	23.7	16.8	10.4	162
Dormancy period	1 Nov–31 March	250	0.46	185	16.5	8.6	2.0	128
Total/annual average		1,218	0.99	354	23.3	15.5	8.0	201
Year 2016								
Budburst-fruit set	1 April–1 June	260	0.95	51	22.7	15.5	7.1	256
Fruit set-veraison	2 June–31 July	363	1.96	0	32.0	23.9	13.8	309
Veraison-harvest	1 August–19 Sept	237	1.71	2	31.9	23.5	14.1	265
Postharvest	20 Sept–31 Octob.	96	0.76	31	25.1	17.5	10.6	159
Dormancy period	1 Nov–31 March	254	0.47	301	16.0	9.3	3.0	119
Total/annual average		1,209	1.01	386	23.0	15.6	7.8	200

prevent severe stress in many wine regions of southern Spain (Resco et al., 2016). One of the main adaptation measures for the vineyards in these areas will be the implementation of strategies, techniques, and technologies that save water and improve the efficiency in the use and application of irrigation water, without causing a decrease in quality. This future scenario with more recurrent drought phenomena and heatwaves will make it more necessary to apply deficit irrigation (DI) techniques as an adaptation to CC. In addition, to face the risks associated with CC and to achieve environmentally sustainable viticulture, vine growers will also have to carry out other changes and make serious adjustments to their traditional production system. These include - as a measure of adaptation in the medium/longer term - the substitution of plant material by different rootstocks, varieties of vine, or clones of the same variety, selected for their better adaptation to and tolerance of the new climatic conditions (Fraga et al., 2013).

The Monastrell variety, native to the Spanish Levant and the most widespread variety in the South East of Spain, is well adapted to these rigorous and dry climates of high temperatures and recurrent drought cycles, because it has evolved since ancient times in these Mediterranean climates. From a physiological point of view, this variety possesses interesting characteristics of drought tolerance, which give it a high capacity to adapt to seasonal water stress and DI strategies (Romero et al., 2010, 2012, 2014). In this respect, we have verified that DI techniques, such as regulated deficit irrigation (RDI) and partial root-zone drying irrigation (PRI), efficiently applied and using moderate annual volumes of water, maintain high yields and improve long-term water use efficiency (WUE) and berry and wine quality in Monastrell wine grapes under semiarid conditions (Romero et al., 2016a, 2016b).

The choice of more efficient and drought tolerant rootstocks has been proposed as another measure of adaptation to CC (Fraga et al., 2013; Berdeja et al., 2015). Genotypic differences in the vigor of the rootstocks induce morphological and anatomical modifications and changes in the distribution of the root system, which may influence the soil volume explored and the availability of water to the plant. In this way, the rootstock can affect the regulation of water relations, (Alsina et al., 2011; Jones, 2012), yield components and fruit composition (Berdeja et al., 2014; Habran et al., 2016).

In a warm winegrowing area of the Jumilla Region (SE Spain), Monastrell vines grafted on an invigorating rootstock (e.g. 1103 P, classified as a rootstock with medium-high drought tolerance and widely used in this area) responded well to the application of different DI techniques - such as PRI and RDI - with moderate amounts of water, the optimum irrigation being 140–150 mm year⁻¹ (Romero et al., 2016a, 2016b). Besides, previous results indicate that distinctive PRI effects (compared to RDI) depend on the volumetric soil water content of the wet root zone (Romero et al., 2012), and that the maintenance of a high water content in the wet root zone during drying-re-watering cycles is critical to improve root-vine performance in long-term PRI wine grapes (Romero et al., 2014, a,b).

Nevertheless, we do not know if this agronomic response can be extrapolated to other soil-climatic conditions and, besides, little is known about the interaction of Monastrell with other rootstocks (of differing vigor and drought tolerance) and with DI techniques such as PRI and RDI, justifying the need for further studies in other climatic conditions, with different rootstocks, DI strategies, and water volumes. Thus, the main goal of this study was to determine if the choice of the rootstock modifies substantially the physiological and agronomic

response of the Monastrell variety to RDI and PRI and to evaluate which of these rootstock-scion combinations is best suited to the application of long-term DI techniques under the semiarid conditions of SE Spain. Thus, five commercial rootstocks (1103 P, 140Ru, 110R, 41B, and 161-49C), on which were grafted mature Monastrell grapevines, were compared under field conditions with the same irrigation volumes and the same controlled DI strategy. Two different irrigation techniques (RDI and PRI) were compared with the aim of distinguishing any specific PRI effects (placement of water). We analyzed the soil-vine water status, root water uptake, vine vigor, fruit growth, leaf mineral nutrition, yield response, WUE, and berry composition in a five-year experiment in a warm and semiarid winegrowing region of SE Spain, taking into account that irrigation will be necessary for vineyard sustainability under semiarid conditions in the future (Resco et al., 2016).

2. Material and methods

2.1. Field conditions, plant materials, and irrigation treatments

This research was carried out from 2012 to 2016 in a 0.4-ha vineyard at the IMIDA experimental station in Cehegín, Murcia, SE Spain (38° 6′ 38.13″N, 1° 40′ 50.41″W, 432 m a. s. l.). The soil was an 80-cm-deep clay loam (33% clay, 38% silt, and 30% sand) with 1.12% organic matter. The irrigation water used came from a well and had a pH of 7.96 and an electrical conductivity of 0.809 dS/m⁻¹. The climate is Mediterranean semiarid, with hot and dry summers and scarce annual rainfall (around 300 mm year⁻¹) (Table 1). The grapevines (*Vitis vinifera* L., var. Monastrell, syn. Mourvedre) were 20+ years-old and were grafted on five different commercial rootstocks: 140Ru, 1103 P, 41B, 161-49C and 110R. The training system was a bilateral cordon trellised to a three-wire vertical system. The vine rows ran NW-SE and the planting density was 2667 vines ha⁻¹, with 3 m between rows and 1.25 m between vines (Fig. 1). Six two-bud spurs (12 nodes) were left after pruning, while in May, green non-productive shoots were removed from each vine in the same manner for all treatments, according to the grower's practice in the area.

Each rootstock-irrigation method combination was drip irrigated during five consecutive years (2012–2016), using two different DI techniques: RDI and PRI. All combinations were irrigated with similar annual water volumes, applying the same designed DI strategy (Table 2): 1) no stress or slight stress from budburst to fruit set; 2) moderate-severe water stress from fruit set to veraison (period of the phenological cycle where grape coloration, its softening and the accumulation of sugars and anthocyanins begins); 3) partial irrigation recovery to maintain moderate stress from veraison to harvest; 4) full recovery postharvest. This DI strategy was initially designed to control excessive vigor during early and mid-season, improve cluster

microclimate, reduce berry size and yield (by using moderate or severe pre-veraison water deficits), and stimulate berry accumulation of sugar, anthocyanins, and other phenolic compounds (by using a moderate post-veraison water deficit). In these periods, the weekly irrigation was modified according to the midday stem water potential (Ψ_s) values (the aim being to maintain moderate levels of Ψ_s , pre-veraison period (fruit set-veraison) and post-veraison period (veraison-harvest) between -1.2 and -1.4 MPa) (Romero et al., 2010, 2013). The final goal of this DI strategy, with low water application and moderate water stress, was to increase WUE and obtain very high quality Monastrell grapes for premium red wine production.

Crop evapotranspiration ($E_{tc} = E_{To} \times K_c$) was estimated using varying crop coefficients (K_c) - based on those proposed by the FAO, adjusted for the Mediterranean area - and reference evapotranspiration (E_{To}) values (Table 1). The K_c values used were 0.35 in April, 0.45 in May, 0.52 in June, 0.75 in July-mid August, 0.60 in mid-August-early September, and 0.45 in mid-September-October. The E_{To} was calculated weekly from the mean values of the preceding 12–15 years, using the Penman Monteith-FAO method (Allen et al., 1998) and the daily climatic data collected in the meteorological station (Campbell mod. CR 10X) located at the experimental vineyard and belonging to the Servicio de Información Agraria de Murcia (SIAM, IMIDA). The experimental design consisted of four replicates per rootstock-irrigation combination in a completely randomized 4-block design. Each replicate contained five vines, only the three central vines being assessed; the border vines in each row were excluded to eliminate potential edge effects.

Irrigation was applied every year from April to October - during the late evening (20:00-23:00), two to five times per week, depending on the phenological period - and was controlled automatically. Each irrigation event lasted between 1 and 3 h, depending on the phenological stage to avoid deep water percolation. The amount of water applied in each irrigation method (PRI and RDI) was measured with flow-meters (model M170, Elster Iberconta, Basque Country, Spain). All rootstock-irrigation method combinations received the same annual, low amount of fertilizers (30 kg N, 20 kg P, 30 kg K, and 16 kg Mg per ha), supplied through the irrigation drip system from April to August. Besides, at the beginning of the growing season a liquid organic matter-amino acid complex was applied. Water was applied by one pressure-compensated emitter per plant (2 L h⁻¹) with one drip-irrigation line per row for the conventional drip irrigation in RDI, and on a double line per row for the PRI with emitters (4 L h⁻¹) to apply the same amount of water in PRI and RDI. In the PRI layout, the two pipelines were joined on both sides of the trunk and located underneath each vine row. In each pipeline in the PRI treatments, there were alternate zones with and without emitters to create dry and wet root zones within each vine row (Fig. 1). In the PRI treatments, water was supplied to only one side of the root system at a time, alternating every 14–16 days. In the RDI treatments,

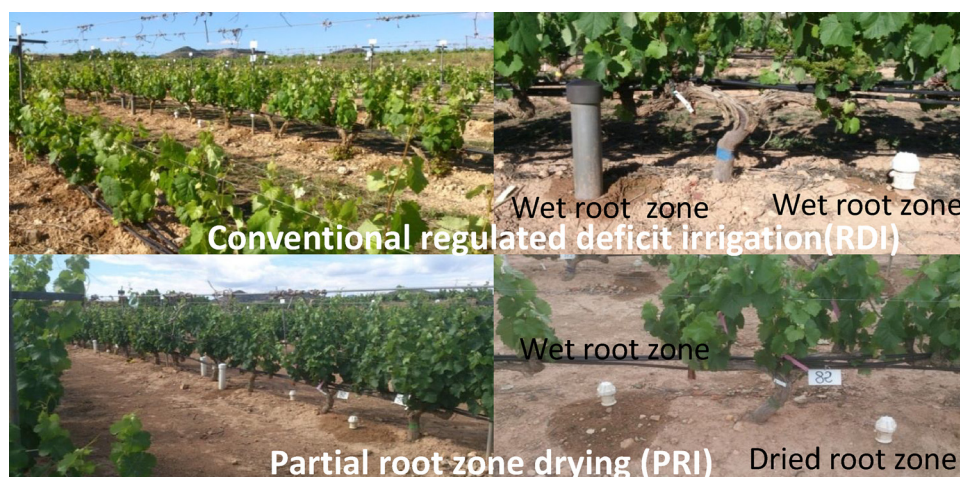


Fig. 1. Application of conventional regulated deficit irrigation (RDI) and partial root zone drying irrigation (PRI) techniques in field-grown Monastrell grapevines grafted onto different rootstocks, showing the two drip lines, soil water content sensors in both root zones, and the wet and dry root zones maintained simultaneously during a typical 14-15-day PRI cycle. After 14 days, the irrigation was changed: the previously dry root zone was irrigated and the previously irrigated root zone was allowed to dry. In each year (2012–2016), 13–14 drying-re-watering PRI cycles were applied during the whole season (April–October).

Table 2

Deficit irrigation strategy and water volume applied for each irrigation method (PRI and RDI) in each phenological period and the total for every year of the experiment (2012–2016).

Year	Irrig. Method	Budburst-Fruitset (mm) April-May	Fruit set-veraison (mm) June-July	Veraison-harvest (mm) Beginning of August-mid September	Postharvest (mm) mid-September-end October	Total annual water volume applied (mm year ⁻¹)
		% ETc (10-20)	%ETc (10)	%ETc (25-30)	%ETc (20-30)	
2012	PRI	19.0	17.8	42.1	7.7	86.7
	RDI	18.3	18.8	43.7	7.8	88.6
2013	PRI	16.6	25.2	37.7	8.6	88.1
	RDI	15.7	24.2	39.2	8.7	87.9
2014	PRI	8.4	26.8	38.6	10.6	84.5
	RDI	8.3	27.1	37.0	10.5	83.0
2015	PRI	22.5	26.7	33.3	8.6	91.1
	RDI	21.2	28.2	32.8	8.7	90.9
2016	PRI	34.7	21.1	29.9	9.6	95.3
	RDI	32.9	21.2	26.3	9.7	90.1
Average (2012–2016)	PRI	20.2	23.5	36.3	9.0	89.1
	RDI	19.3	23.9	35.8	9.1	88.1

irrigation water was supplied simultaneously to the entire root system. We applied 13–14 drying-re-watering PRI cycles during the whole season (from early-mid April to the end of October) every year.

2.2. Soil water content, root dry biomass, and root water uptake rate

From 2013 to 2016 the volumetric soil water content (θ_v) was generally measured between 09:00 and 10:00 (always 10–12 h after irrigation), three times per week (daily in specific periods in 2014 and 2015), with a Diviner 2000 portable soil moisture probe (Sentek Pty Ltd., Stepney, SA, Australia). PVC access tubes were installed to a depth of 100 cm in one (RDI) or both (PRI) parts of the root zone, 60 access tubes in total. Readings were taken close to the vines, 10–15 cm from the drip head and oriented perpendicularly to the drip lines (Fig. 1), at depths from 10 to 100 cm (maximum depth) for four replicates per rootstock-irrigation method combination (one per plot). At the beginning of the experiment (2012) a soil texture analysis was performed in different soil profiles (45 soil samples) in the whole experimental vineyard. Scaled frequency (SF) values were converted to θ_v using a capacitance probe calibration equation ($SF = 0.2352\theta_v^{0.3672}$) for clay loam soil (Paraskevas et al., 2012), of similar texture to our vineyard soil.

In order to estimate the soil zone with the highest fine root density (with higher soil water and nutrient uptake capacity), root samples were collected at the end of the experimental period (summer 2016) in soil cores (100 cm³), using an auger. The samples were taken close to the vines, perpendicularly to an emitter at 10–15 cm from the drip head (in the wet root zone), at 20-cm intervals to a depth of 100 cm, for two vines per rootstock (one in PRI and one in RDI). The roots were washed and shaken in water to separate fine roots from soil particles, filtered, classified in class diameters, and dried in an oven at 80 °C for 24–48 h. The results were used in the root water uptake study, described below.

In 2014 and 2015 the root water uptake rates ($\Delta\theta_v \Delta t^{-1}$, mm day⁻¹) of different soil profiles (10–50 cm and 60–80 cm) were estimated, using the discrete readings of soil moisture probes to determine the changes in θ_v with time between consecutive days with irrigation (R) and no irrigation (NR) and between consecutive days with no irrigation (NR-NR). Root water uptake was estimated using methodology similar to that used by IrriMax® version 9.1.1 Sentek (2012) software for continuous θ_v FDR measurements (EnviroSCAN, Sentek Pty Ltd), adapted for discrete FDR measurements. We considered the variations of the water stock in the soil profile studied (depth 10–50 cm, zone of highest root density) and in the profile immediately below (60–80 cm), according to the expression:

$$Abs_w^{10-50} = \begin{cases} [\Delta\theta_v]_{10-50} & [\Delta\theta_v]_{60-80} < 0 \\ [\Delta\theta_v]_{10-50} - [\Delta\theta_v]_{60-80} & [\Delta\theta_v]_{60-80} > 0 \end{cases}$$

Abs_w^{10-50} ($\Delta\theta_v \Delta t^{-1}$, mm day⁻¹) is the net water uptake rate in the soil profile (10–50 cm depth) between two consecutive days (R-NR and NR-NR); $[\Delta\theta_v]_{10-50}$ is the gross variation of the water stock in the soil profile (10–50 cm) between two consecutive days. For this, we used discrete measurements: 10, 20, 30, 40 and 50 cm to calculate water stock. $[\Delta\theta_v]_{60-80}$ is the gross variation of the water stock in the soil profile immediately below (60–80 cm depth, for which we have data) between two consecutive days. For this, we used discrete measurements: 60, 70 and 80 cm to calculate water stock. When $[\Delta\theta_v]_{60-80} > 0$ this indicates that water is reaching that profile by percolation and must be removed from the $[\Delta\theta_v]_{10-50}$ observed. The Abs_w^{60-80} was calculated in a similar way, but taking into account the $[\Delta\theta_v]_{90-100}$. The variation of the water stock in a soil profile (a, b) between two consecutive days (1, 2) is given by the following equation (Romero et al., 2012):

$$[\Delta\theta_v]_{a,b} = \left[\sum_a^b \theta_v \right]_1 - \left[\sum_a^b \theta_v \right]_2, \text{ mm day}^{-1}$$

2.3. Vine water status and leaf photosynthesis

Each year, the stem water potential (Ψ_s) was determined weekly or fortnightly from the beginning of vegetative growth until leaf fall. Six to ten healthy, fully exposed and expanded mature leaves from the main shoots in the middle-upper part of the vine canopy were taken per rootstock. The leaves were enclosed in aluminum foil and covered with plastic at least 2 h before the midday measurement. The Ψ_s was measured at noon (12:00–13:30) using a pressure chamber (Model 600; PMS Instrument Co., Albany, OR, USA). During some important periods (from fruit set to veraison and from veraison to harvest), Ψ_s was measured more frequently (twice a week: at the beginning and end of the week, and before and after an irrigation event). The water stress integral (S_{Ψ} , MPa x day) was calculated for the years 2012, 2013, and 2015 using the midday xylem water potential data, according to the methodology defined by Myers (1988).

Net leaf photosynthesis was measured between 09:00 and 10:30 every 7–14 days from May to October in 2013, 2014, and 2015, on selected clear and sunny days. Measurements were made on healthy, fully expanded, mature leaves exposed to the sun (one leaf on each of 8 or 16 vines per rootstock, depending on the year) and growing on main shoots located on the exterior canopy. Leaf photosynthesis rates in the early morning (A) were measured with a portable photosynthesis measurement system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with

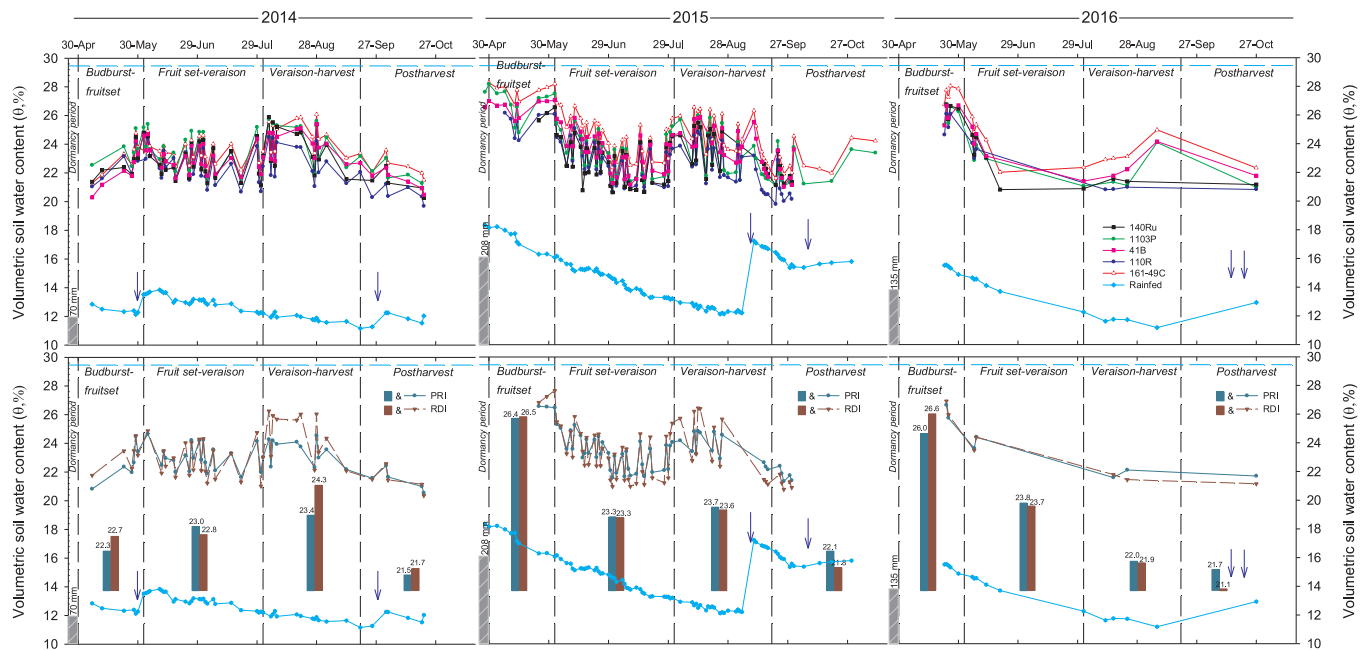


Fig. 2. Seasonal evolution of mean values of volumetric soil water content (θ_v) in the soil-profile (10–50 cm depth) for different rootstocks (161-49C, 1103 P, 110R, 140Ru, and 41B) (above) and irrigation methods: RDI and PRI (below), in three consecutive years of the experimental period (2014–2016). Cyan lines represent the θ_v maintained for vines under rain-fed conditions every year and green dotted lines represent the field capacity (FC) of the experimental soil. Gray vertical bars represent the cumulative rainfall during the dormancy period (November–April) for each year. Blue arrows represent rainfall events exceeding 15 mm. Colored vertical bars with values represent the mean values of θ_v maintained during different phenological periods in each irrigation system (PRI and RDI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

a broadleaf chamber (6.0 cm²). During measurements, the leaf temperature was between 23 °C and 39 °C, the leaf-to-air vapor pressure deficit was between 1.4 and 5.3 kPa, and the relative humidity was 30–50%. The molar air flow rate inside the leaf chamber was 500 $\mu\text{mol mol}^{-1}$. All measurements were taken at a reference CO₂ concentration similar to ambient (390 $\mu\text{mol mol}^{-1}$) and at a saturating PPFD of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.4. Leaf mineral analysis

Leaf samples were collected in July 2013 and 2014 for mineral analysis. About 40 leaves were collected from eight vines per treatment. They were washed immediately, dried at 65 °C for one week, and milled. After the plant tissue was digested, the ashes were dissolved in HNO₃ and K, Mg, Ca, Na, P, Fe, Mn, Zn, and B were analyzed with an inductively coupled plasma optical emission spectrometer (Varian MPX Vista, Palo Alto, CA). The nitrogen concentration was determined with a LECO FP-428 protein detector.

2.5. Vine vigor parameters and aboveground fresh matter accumulation

The changes in trunk diameter of 16 vines per rootstock (4 vines per plot) were measured monthly during the experimental period (from April to October in 2014 and 2015) using a permanent girth measurement system (D1 dendrometer, UMS, Meter Group AG, Munich, GE) located in the trunk at 20 cm above the soil surface. The terminal growth of one main shoot (East-oriented) per vine, in 16 vines per rootstock (four vines per plot), was measured weekly or fortnightly from early April to the end of July. The total shoot length was measured with a tape measure. The total number of main shoots per vine was also recorded each year, at the end of June, in the same vines. Besides, in 2014 and 2015, in one representative main shoot per vine (16 vines per rootstock), the total number of lateral shoots was counted and the lateral shoot length growth was measured weekly, using a tape measure, in previously labeled lateral shoots.

From 2012 to 2015, the total leaf area (TLA) per vine was estimated pre- and post-veraison, in 8 or 16 vines per rootstock (depending on the year), using a non-destructive method: a first order polynomial equation was developed for each rootstock, relating main shoot length (SL) to total leaf area (TLA) of the main shoot. The TLA per plant was calculated by selecting four or five representative main shoots per vine and calculating their average SL. The TLA per plant was estimated by multiplying the average shoot leaf area by the total number of main shoots of the vine. To develop the polynomial equations, in 2013 initially, from early May to the end of June, we selected randomly between 34 and 38 main shoots of different lengths per rootstock and the leaf area per main shoot (main and lateral leaves) was measured using a leaf area meter (Licor 3000, Li-Cor, Lincoln, NE, USA). The linear equations used were: 110R: $\text{TLA (cm}^2\text{)} = -1227 + 37.3\text{SL}$, $r = 0.96^{***}$; 140Ru: $\text{TLA (cm}^2\text{)} = -1985 + 45.05\text{SL}$, $r = 0.92^{***}$; 161-49C: $\text{TLA (cm}^2\text{)} = -1915 + 46.1\text{SL}$, $r = 0.95^{***}$; 41B: $\text{TLA (cm}^2\text{)} = -2014 + 45.6\text{SL}$, $r = 0.94^{***}$; 1103 P: $\text{TLA (cm}^2\text{)} = -2267 + 49.4\text{SL}$, $r = 0.95^{***}$.

The exposed leaf area was estimated every year (2012–2015) during the pre- and post-veraison periods, in 16 vines per treatment (the same vines used for TLA and shoot growth measurements), by measuring the external perimeter of the leaf area (the height and width of the canopy). The distance from the beginning of the leaf layer on one side of the vine (East) to the end of the leaf layer on the other side (West) was measured using a tape measure. Four measurements per vine were made. Canopy gaps were corrected for by applying a reduction of 5% (Martínez de Toda, 2008). To calculate the exposed leaf area per vine, the average of four external perimeter measurements (m) was multiplied by the distance between vines (1.25 m).

In 2013, 2014, and 2015, the percentage of healthy leaves (not infected by pathogens, nor with nutritional deficiencies) per vine was estimated visually at the end of growing period, in September and October, for 24 vines per rootstock (12 in each irrigation method). In the winters of the five years, the pruning weight was measured for 24 vines per rootstock (including the same vines from which the trunk growth and shoot measurements were taken).

The total fresh biomass aboveground per vine (including fruits,

Table 3

Root water uptake rates ($\Delta\theta$, Δt^{-1} , mm H₂O day⁻¹) estimated for each rootstock, irrigation method (PRI and RDI), and year and the interaction rootstock (R) x irrigation method (IM), calculated for each phenological period and as the average annual value in the shallow soil profile (10–50 cm depth). Data from two years (2014 and 2015).

	Budburst- fruit set	Fruit set- veraison	Veraison- harvest	Postharvest	Annual
Rootstock (R)					
140RU	6.66 b	6.71 b	10.24 c	3.54	6.77 b
1103 P	7.72 b	7.34 b	12.46 d	4.72	8.06 b
41B	4.53 a	5.19 a	6.91 a	3.57	5.04 a
110R	6.47 b	7.37 b	9.44 bc	3.74	6.76 b
161-49C	4.10 a	5.11 a	7.87 ab	2.79	4.97 a
Irrigation method (IM)					
PRI	5.02	4.86	6.84	2.97	4.87
RDI	6.77	7.82	11.93	4.38	7.77
Year					
2014	5.79	6.05	10.09	4.06	6.50
2015	6.01	6.64	8.68	3.29	6.14
Interaction (R x IM)					
140RU PRI	–	4.91	7.47	2.50	4.96
140RU RDI	–	8.51	13.00	4.59	8.58
1103 P PRI	–	5.85	8.33	3.61	6.02
1103 P RDI	–	8.82	16.59	5.83	10.10
41B PRI	–	4.40	4.67	3.19	4.08
41B RDI	–	5.98	9.16	3.96	5.99
110R PRI	–	4.94	7.08	2.69	4.88
110R RDI	–	9.80	11.80	4.80	8.64
161-49C PRI	–	4.21	6.65	2.86	4.41
161-49C RDI	–	6.02	9.09	2.72	5.52
ANOVA					
R	***	**	***	ns	***
IM	**	***	***	**	***
Year	ns	ns	*	ns	ns
R x IM	–	ns	ns	ns	ns

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level. The symbol – means that there were not enough data to calculate the interaction.

main and lateral shoots, and main and lateral leaves) was reported in 2014 and 2016. At the end of August-early September, 8–10 vines (not used for yield) were selected for each rootstock (4–5 for each irrigation method) and one or two representative main shoots per plant were taken. All leaves from the main shoots (including from the lateral shoots) were collected and the fresh and dry weights (65 °C until constant weight) were determined. The fresh and dry weights of the main and lateral shoots (without leaves) and clusters were also measured and the water content of the shoots, leaves, and clusters was calculated. The number of main shoots and clusters per vine was also counted.

2.6. Fruit growth and development

In 2013, 2014, and 2015, during berry development (from early June to mid-September), 24–48 berries per rootstock (depending on the year) that had been labeled previously in intact sun exposed clusters were used to make fortnightly, non-destructive berry diameter measurements, with a Mitutoyo digital caliper (MTI, City of Industry, CA). Absolute (AGR) and relative growth rates (RGR) of berry diameter were calculated. In 2014 and 2015, during the ripening period (from the end of July or early August until harvest), 8 to 16 berry samples (15 berries each) were collected from 8 or 16 selected vines per rootstock (in both irrigation methods) every 10–15 days, depending on the year. Total soluble solids (TSS, °Brix), fresh weight, and dry weight were measured and the °Brix, total solutes per berry, water, and fresh and dry weight accumulation rates were determined.

2.7. Yield response, sink/source ratios, and efficiency indices

In 2014 and 2015, the number of total buds per vine before and after budburst (number of buds broken after budburst), the number of main shoots with clusters, and the number of clusters per shoot were counted and bud fruitfulness and % fructification (number of main shoots with clusters vine⁻¹/ number of total main shoots vine⁻¹) were calculated. Each year at harvest, the yield components were measured for 24 vines per rootstock (12 vines per irrigation method). The harvest date was in accordance with the grower's practice in the area, when °Brix reached 23.5–24.0. The yield per vine, number of clusters per vine, cluster weight, berry number per cluster, and berry weights were calculated. In 2013 and 2015 the degree of compactness of clusters (1 meaning low compactness and 5 high compactness) and the cluster size (big clusters, length > 20 cm; medium clusters, 15 < length < 20 cm; and small clusters, length < 15 cm) were determined in 24 vines per rootstock (12 for each irrigation method) at the end of ripening.

The vegetative growth and productive data of the different years were used to calculate several vine vigor indices and sink/source ratios, such as yield/pruning weight, TLA/yield, exposed leaf area/TLA, and the exposed leaf area/yield/shoot length index - a new viticultural index, referred to as the Toda index (Martínez de Toda et al., 2007). Also, several efficiency indices were calculated. The productive water use efficiency (WUE_{yield}) was expressed as the mass of fresh grapes produced per m³ of applied water, per vine, the DW_{yield} as kg of annual dry fruit biomass produced per m³ of applied water, and the fresh biomass water productivity (FW_{productivity}) as kg of total annual fresh biomass produced aboveground per m³ of applied water. The DW_{productivity} was calculated as kg of total annual dry biomass produced aboveground per m³ of applied water and the harvest index (HI, expressed as FW or DW) as the proportion of total annual fresh or dry biomass produced (aboveground) that actually ended up in the harvested product (Hsiao et al., 2007).

2.8. Berry quality

Samples of mature berries were collected from each grapevine in September 2012, 2013, 2014, 2015, and 2016, when maturity was around 23.5–24° Brix (coinciding with harvest), and transported to the laboratory. The harvest dates were determined on the basis of weekly analyses of the grape composition. The samples consisted of 800–900 g of berries, randomly collected from different clusters in each vine. The berries were crushed by an automatic blender (Coupe 550 GT), avoiding seed breakage. A first sub-sample of this crushed sample of grapes was centrifuged and the juice was used for the analysis of pH, TSS, solutes per berry (g), titratable acidity, and organic acids (tartaric and malic), as described in detail in Romero et al. (2013, 2015). A second sub-sample was kept frozen (–80 °C) until analysis of resveratrol, piceid, flavonoids, amino acids, and antioxidant activity.

The phenolic potential of the grapes was calculated according to the method described by Saint-Cricq et al. (1998) and Ribéreau-Gayon et al. (2006).

We calculated the berry quality indices (QIs) described by Romero et al. (2016c) in Monastrell grapevines, with some modifications. Briefly, the berry technological quality index (QI_{technological berry}) was calculated following the same criteria as Romero et al. (2016c) – except for °Brix, which was modified to give a score of zero for °Brix > 25. For the berry phenolic quality index (QI_{phenolic berry}), we assigned a score of two when extractable polyphenols were between 55 and 70.

Resveratrol and piceid were extracted with ethyl acetate, as described by Ribeiro de Lima et al. (1999). Analysis was carried out by HPLC-MS/MS (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA) with an ESI interface operating in negative ion mode, using the following operation parameters: 4000 V capillary voltage, 60 psi nebulizer pressure, 11 L/min drying gas flow, and 350 °C drying gas temperature. Separation was achieved in a Lichrosphere C₁₈ analytical

Table 4

Average values of Ψ_s , midday stem water potential (MPa), and A_n , early-mid-morning leaf net photosynthesis rate measured early-mid morning ($\mu\text{mol m}^{-2} \text{s}^{-1}$), during different pre- and post-veraison periods for each rootstock (R), irrigation method (IM), and year, and the interaction (R x IM). The Ψ_s data are from a 3–5 year period (2012–2016) and the leaf photosynthesis data are from three years (2013, 2014, and 2015).

Midday stem water potential (Ψ_s) (MPa)							Maximum net leaf photosynthesis (A_n) ($\mu\text{mol m}^{-2} \text{s}^{-1}$)						
	Budburst- Fruit set	Fruit set- veraison	Veraison- harvest	Early ripening (2- 23 August)	Post-harvest	Annual entire cycle	Budburst- Fruit set	Fruit set- veraison	Veraison- harvest	Early ripening (2- 23 August)	Post- harvest	Annual entire cycle	
Rootstock (R)													
140Ru	−0.59	−0.98c	−1.13b	−1.19d	−0.94b	−0.90c	13.0	16.7d	14.2c	15.1c	8.7b	14.6c	
1103 P	−0.60	−1.09a	−1.22a	−1.24bb	−1.08a	−1.00a	13.0	14.6a	12.3bc	13.2bc	7.5a	12.9a	
41B	−0.58	−1.08a	−1.22a	−1.28ab	−1.05a	−0.98a	12.2	15.3cb	12.2bc	12.6a	8.6b	13.1a	
110R	−0.60	−1.00c	−1.14b	−1.22cd	−0.99ab	−0.93b	13.0	15.8c	12.8b	13.7b	8.7b	13.6b	
161-49C	−0.59	−1.03b	−1.25a	−1.30a	−1.07a	−0.99a	12.2	15.1b	11.8a	12.7a	6.9a	12.8a	
Irrigation method (IM)													
PRI	−0.59	−1.04	−1.22	−1.26	−1.07	−0.97	12.9	15.4	12.6	13.3	7.9	13.3	
RDI	−0.60	−1.04	−1.17	−1.23	−0.98	−0.94	12.5	15.6	12.8	13.7	8.3	13.5	
Year													
2012	−0.59ab	−1.03bc	−1.29a	−1.35a	−1.15a	−1.02b	–	–	–	–	–	–	
2013	−0.48b	−0.89d	−1.12c	−1.17c	−1.05b	−0.88d	13.5b	17.2c	12.5b	13.4b	8.6b	13.6b	
2014	−0.79a	−1.20a	−1.15bc	−1.21bc	–	−1.04a	13.8b	15.9b	14.1c	15.5c	–	14.9c	
2015	−0.51b	−1.05b	−1.17b	−1.22b	−0.87c	−0.90c	10.9a	13.4a	11.4a	11.6a	7.6a	11.8a	
2016	–	−1.02c	–	–	–	–	–	–	–	–	–	–	
Interaction (R x IM)													
140Ru	PRI	−0.58	−0.98	−1.14	−1.19	−0.96	−0.91fg	13.2	16.5f	14.0	14.7	8.4	14.4
	RDI	−0.59	−0.99	−1.12	−1.19	−0.91	−0.89g	12.9	16.9f	14.4	15.5	9.1	14.7
1103 P	PRI	−0.59	−1.10	−1.27	−1.27	−1.18	−1.03a	13.6	14.7ab	12.5	13.2	7.4	13.0
	RDI	−0.62	−1.09	−1.18	−1.21	−0.98	−0.97cd	12.5	14.5a	12.2	13.3	7.7	12.8
41B	PRI	−0.58	−1.08	−1.23	−1.29	−1.05	−0.99bc	12.3	15.7de	12.4	12.9	8.5	13.4
	RDI	−0.58	−1.08	−1.20	−1.27	−1.04	−0.98c	12.1	14.8abc	12.1	12.3	8.7	12.9
110R	PRI	−0.60	−1.01	−1.17	−1.23	−1.03	−0.94de	13.5	15.3bcd	12.4	13.2	8.6	13.4
	RDI	−0.61	−0.99	−1.12	−1.21	−0.96	−0.93ef	12.6	16.2ef	13.1	14.1	8.8	13.9
161-49C	PRI	−0.60	−1.02	−1.27	−1.33	−1.12	−1.01ab	11.9	14.7ab	11.6	12.3	6.7	12.5
	RDI	−0.58	−1.03	−1.22	−1.28	−1.01	−0.97cd	12.5	15.5cde	12.0	13.1	7.1	13.0
ANOVA													
R	ns	***	***	***	***	***	ns	***	***	***	***	***	
IM	ns	ns	**	*	***	***	ns	ns	ns	ns	ns	ns	
Year	***	***	***	***	***	***	***	***	***	***	***	***	
R x IM	ns	ns	ns	ns	ns	*	ns	**	ns	ns	ns	ns	

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

column of 250 mm × 4 mm and 5 μm particle size (Agilent Technologies, Waldbronn, Germany), with 5% formic acid in water (solvent A) and acetonitrile (solvent B) as the mobile phase at a flow rate of 0.8 mL min^{−1}. Keracyanin was used as internal standard. Selective reaction monitoring (SRM) transitions (two per compound) were optimized using several fragment or voltages (F), from 20 to 200 V, and collision energies (CE) from 2 to 50 V. The MS/MS transitions were m/z 389 → 227 and 389 → 185 for piceid, and 227 → 185 and 227 → 143 for resveratrol. Resveratrol and piceid (*cis* and *trans* isomers) were quantified with respect to their corresponding standards purchased from Sigma-Aldrich (Steinheim, Germany).

One gram of grape was extracted with 9 mL of 80% methanol for 30 min at room temperature. After centrifugation at 5000 g for 10 min, the supernatant was used for determination of total flavonoids and antioxidant capacity. The content of total flavonoids was measured according to Dewanto et al. (2002), with the modifications of Tounsi et al. (2011). The test used to determine the antioxidant capacity of the fruit juice was the ABTS⁺ radical cation assay, using Trolox to standardize the system (Miller et al., 1993, 1996) with some modifications (Navarro et al., 2006).

The amino acids were extracted from 500 mg of grape with 80% ethanol, three consecutive times. Each combined extract was evaporated to dryness and the residue was dissolved in deionized water and filtered through a Sep-Pak C18 cartridge. The extract was used for amino acids determination by the ninhydrin method (Rosen, 1957).

2.9. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) procedures and means were separated by Duncan's multiple range test, using Statgraphics 2.0 Plus software (Statistical Graphics Corporation, USA). A three-way ANOVA procedure was used to discriminate the effects of rootstock, irrigation method, and year. Linear and nonlinear regressions were fitted using SigmaPlot 11.0 (Systat, Richmond, CA). Schwarz's Bayesian criterion index (SBC) was used to find the best fit for nonlinear regression between parameters.

3. Results

3.1. Soil moisture and root water uptake

The volumetric soil water content (Θ_v), averaged for all rootstocks, was above 20% during the entire season in shallow layers (10–50 cm depth) (Fig. 2) and higher (> 22%) in deeper soil layers (60–100 cm) (Fig. 1S, Supporting information). For the vines growing under rain-fed conditions a low seasonal Θ_v was maintained at 10–50 cm depth, around 12–14%, except in certain periods when there was some re-charge of water in the soil profile due to intense rainfall events ($\Theta_v = 18\%$). In addition, the Θ_v in deeper soil layers (60–100 cm depth) changed little during most of the growing season, indicating minor deep percolation, according to the low irrigation volumes applied (Fig. 1S).

Regardless of the rootstock, the irrigation method (PRI vs. RDI) had

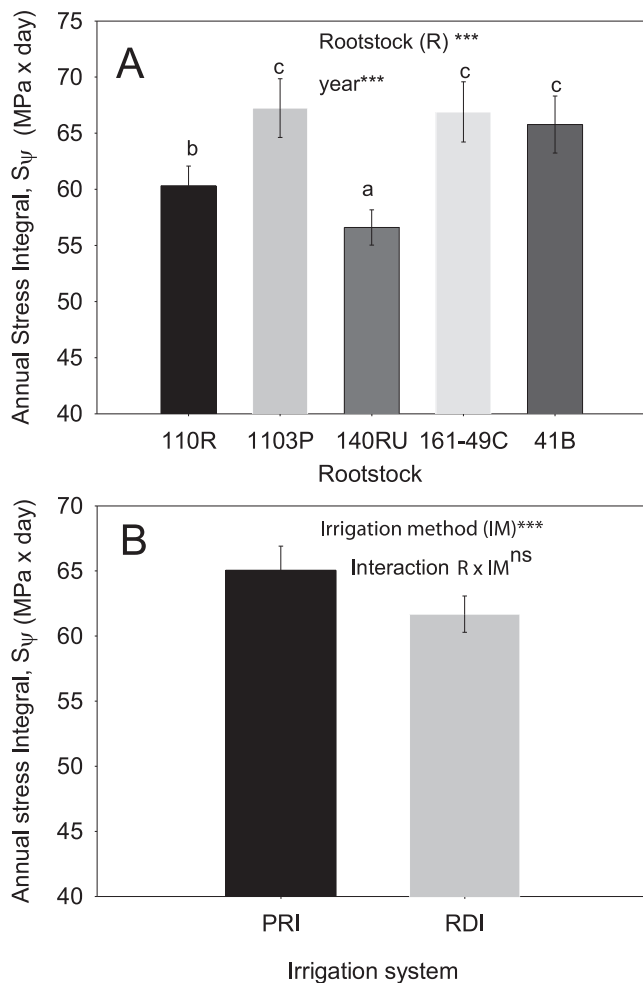


Fig. 3. Mean values of the annual water stress integral (S_{ψ}) for each rootstock (A) and irrigation method (B). Average of three years (2012, 2013, and 2015). Vertical bars represent the standard errors. ns, not significant; ***, $P < 0.001$. Separation was by Duncan's multiple range test at the 95% confidence level.

a significant effect on the averaged seasonal Θ_v calculated for the three years, especially in some periods (budburst-fruit set and veraison-harvest). In general, PRI vines had a significantly ($P < 0.05$) lower Θ_v (averaged for dry and wet root zones) at 10–50 cm depth than RDI vines, but a higher Θ_v at 60–100 cm depth (Fig. 2, Fig. 1S), indicating deeper water percolation in PRI. The effect of the year was also significant, 2014 having the lowest averaged Θ_v , reflecting the drier and warmer conditions (Table 1) compared to other, more rainy years (2015 and 2016).

The seasonal root water uptake ($\Delta\Theta_v \Delta t^{-1}$) at 10–50 cm depth shows that vines grafted on rootstocks 41B and 161-49C had lower root water uptake rates than those on the other rootstocks (110R, 140Ru, and 1103P) during most of the growing season (budburst-harvest) (Table 3). However, in deeper soil (60–80 cm), vines on 41B maintained significantly higher seasonal $\Delta\Theta_v \Delta t^{-1}$ values than those on the other rootstocks (Table 1S, Supporting information). In general, for all rootstock-irrigation method combinations, most of the root water uptake (between 85%, for 41B, and 96%, for 1103P) occurred in the first 50 cm of soil depth, the zone with the highest root ($\Phi < 5$ mm) density (Fig. 2S, Supporting information). In deeper soil (60–80 cm), $\Delta\Theta_v \Delta t^{-1}$ only accounted for between 4% (1103P) and 15% (41B) of total uptake (Table 3, Table 1S). Besides, $\Delta\Theta_v \Delta t^{-1}$ was clearly highest during the veraison-harvest period, in all rootstocks, coinciding with berry ripening (from late July-early August to mid-September), when the evaporative demand of the atmosphere and plant water needs (Table 1)

were very high. During this period, vines grafted on 1103P maintained the highest averaged $\Delta\Theta_v \Delta t^{-1}$ (significantly higher than the rest of the rootstocks), followed by vines grafted on 140Ru (Table 3).

In general, for all rootstocks, PRI vines maintained significantly lower $\Delta\Theta_v \Delta t^{-1}$ values in surface soil layers (10–50 cm), during the entire growing season, compared to vines under RDI (Table 3), although this effect was not observed in deeper layers (Table 1S).

3.2. Vine water status and individual leaf photosynthesis

Vines grafted on rootstocks 140Ru or 110R maintained, during the whole season, a significantly better (less negative Ψ_s) vine water status than those on 1103P, 41B, or 161-49C (Table 4). Vines grafted on 161-49C experienced the most severe water stress during early ripening and veraison-harvest period (Table 4), and they had the highest annual averaged S_{ψ} (together with 1103P and 41B, Fig. 3). Besides, vines grafted on 1103P and 41B experienced greater water stress from fruit set to veraison ($P < 0.05$) than those on the other rootstocks (Table 4). The seasonal evolution of Ψ_s indicates that for most of the growing season (especially from fruit set to harvest) Ψ_s was maintained within a range of values indicating moderate water stress (between -1.25 and -1.35 MPa) for all rootstocks, and only occasionally, in a short period of 2012 for 41B and 1103P, exceeded the critical threshold of -1.4 MPa (Fig. 3).

Also, according to the higher S_{ψ} values for PRI vines (Fig. 3), and regardless of the rootstock, PRI vines experienced greater water stress during the veraison-harvest (especially at early ripening) and post-harvest periods than RDI vines (Table 4, Fig. 3). In addition, significant interactive effects were observed for the annual averaged Ψ_s , with PRI vines grafted on 1103P and 161-49C showing lower Ψ_s than RDI vines (Table 4). The years with the most severe vine water stress were 2012 and 2014, while the stress was milder in 2013 and 2015.

Vines grafted on 140Ru maintained significantly higher net leaf photosynthesis measured at early-mid morning (A_n) during most of the growing season (especially from fruit set to harvest), followed by vines grafted on 110R (Table 4). Differences in averaged A_n of up to $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ were observed in some phenological periods between vines grafted on 140Ru (the maximum A_n) and vines grafted on 161-49C and 1103P, which maintained the lowest values of seasonal A_n . The year also had a significant effect on leaf photosynthesis, A_n reaching its maximum levels in 2014 and its minimum in 2015.

Although in general there were no significant effects of the irrigation method on A_n , a significant interaction between the rootstock and irrigation method was observed during the fruit-set-veraison period. In this period PRI vines grafted on 161-49C or 110R had lower averaged A_n values than RDI vines, while PRI vines grafted on 41B maintained significantly higher A_n values than RDI vines (Table 4).

3.3. Leaf mineral nutrition

The rootstock and the year had an important effect on leaf mineral composition. In general, invigorating rootstocks (140Ru and 1103P) enhanced significantly the leaf N, P, and Mg (1103P) contents and decreased B, compared to low vigor rootstocks (161-49C and 110R) (Table 5). The irrigation method only had a significant effect on the leaf Zn and B contents, with PRI vines showing significantly lower Zn and higher B than RDI vines (Table 5). Significant interactive effects of the rootstock and irrigation method were observed for N, P, Ca, Mg, and Mn (Table 5).

3.4. Fruit growth and ripening

The RGR of berries on the rootstock 140Ru was significantly higher than for the rest of the rootstocks, mainly during the post-veraison periods (Table 6). Conversely, the RGR of 1103P berries showed the lowest value. In addition, during pre-veraison in 2014 and 2015, the

Table 5

Leaf nutrient concentrations of Monastrell grapevines at veraison, for five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C) and two different irrigation methods (PRI and RDI). The N, P, K, Ca, Mg, and Na are expressed as % in DW and Fe, Mn, Zn, and B as ppm. The data are from 2013 and 2014.

	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	B
Rootstock (R)										
140Ru	2.95b	0.142c	0.51	1.58a	0.36a	0.07	72	84ab	28.2	50b
1103 P	2.89b	0.142c	0.53	1.74bc	0.44c	0.08	78	86ab	30.3	52b
41B	2.79a	0.136b	0.56	1.69ab	0.38ab	0.07	77	70a	29.7	42a
110R	2.80a	0.147c	0.54	1.70ab	0.40b	0.10	81	97b	32.8	61c
161-49C	2.75a	0.129a	0.50	1.82c	0.38ab	0.08	73	79ab	30.2	66c
Irrigation method (IM)										
PRI	2.84	0.138	0.53	1.67	0.39	0.08	75	85	28.8	59
RDI	2.83	0.140	0.53	1.74	0.40	0.08	77	81	31.7	50
Year										
2013	2.69	0.142	0.65	2.13	0.39	0.06	73	96	40.5	54
2014	2.98	0.136	0.41	1.28	0.40	0.10	80	69	20.0	55
Interaction (R x IM)										
140Ru PRI	2.88cd	0.137bc	0.52	1.67bc	0.38abc	0.08	72	90de	27.6	52
140Ru RDI	3.01e	0.148d	0.50	1.48a	0.35a	0.06	72	77bc	28.9	47
1103 P PRI	2.93de	0.144cd	0.51	1.61ab	0.42c	0.08	74	88cde	29.0	55
1103 P RDI	2.85bcd	0.141bcd	0.55	1.86d	0.46d	0.07	82	83bcd	31.6	49
41B PRI	2.81abc	0.138bc	0.56	1.58ab	0.36ab	0.09	79	64a	28.5	47
41B RDI	2.76abc	0.133b	0.56	1.80cd	0.40bc	0.06	75	76b	30.9	38
110R PRI	2.85bcd	0.149d	0.56	1.66abc	0.40bc	0.09	79	96e	31.1	63
110R RDI	2.74ab	0.144cd	0.52	1.73bcd	0.39abc	0.10	83	97e	34.5	60
161-49C PRI	2.71a	0.122a	0.48	1.83cd	0.39abc	0.07	72	85bcde	27.9	76
161-49C RDI	2.80abc	0.136bc	0.52	1.81cd	0.38abc	0.09	75	73ab	32.5	57
ANOVA										
R	***	***	ns	**	***	ns	ns	***	ns	***
IM	ns	ns	ns	ns	ns	ns	ns	ns	**	***
Year	***	**	***	***	ns	***	*	***	***	ns
R x IM	*	**	ns	**	*	ns	ns	*	ns	ns

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

Table 6

Relative growth rate (RGR, mm mm⁻¹ day⁻¹) of berries from different rootstocks, irrigation methods (PRI and RDI) and their interactions during the pre- and post-veraison periods in 2013, 2014, and 2015. Berry/trunk growth ratios calculated for pre- and post-veraison in 2014 and 2015.

			RGR berry			Berry/trunk growth ratio	
	Pre-veraison 2013	Post -veraison	Pre-veraison 2014	Pre-veraison 2015	Post-veraison	Pre-veraison 2014–2015	Post-veraison 2015
Rootstock (R)							
140Ru	0.0091	0.0127b	0.0110	0.0065	0.0025b	6.02a	8.96
1103 P	0.0096	0.0093a	0.0121	0.0067	0.0015a	7.02a	4.22
41B	0.0115	0.0109ab	0.0119	0.0061	0.0017a	6.07a	8.17
110R	0.0095	0.0095a	0.0126	0.0060	0.0020ab	14.85b	10.63
161-49C	0.0105	0.0107ab	0.0116	0.0067	0.0021ab	11.72ab	7.54
Irrigation method (IM)							
PRI	0.0099	0.0107	0.0114	0.0062	0.0019	9.10	7.91
RDI	0.0102	0.0105	0.0123	0.0066	0.0020	9.17	7.90
Interaction (R x IM)							
140Ru PRI	0.0085	0.0131	0.0113	0.0059	0.0030c	6.63	11.32
140Ru RDI	0.0097	0.0124	0.0108	0.0072	0.0020abc	5.41	6.61
1103 P PRI	0.0091	0.0097	0.0111	0.0067	0.0013a	5.09	3.56
1103 P RDI	0.0101	0.0088	0.0131	0.0067	0.0016ab	8.96	4.88
41B PRI	0.0116	0.0096	0.0113	0.0059	0.0021abc	5.23	11.31
41B RDI	0.0113	0.0121	0.0127	0.0063	0.0013a	6.90	5.02
110R PRI	0.0104	0.0092	0.0128	0.0064	0.0013a	17.83	7.13
110R RDI	0.0086	0.0097	0.0121	0.0057	0.0027bc	11.87	14.14
161-49C PRI	0.0097	0.0121	0.0105	0.0061	0.0019ab	10.70	6.24
161-49C RDI	0.0113	0.0094	0.0126	0.0073	0.0022abc	12.73	8.83
ANOVA							
R	ns	*	ns	ns	*	*	ns
IM	ns	ns	*	*	ns	ns	ns
R x IM	ns	ns	ns	ns	**	ns	ns

'ns' not significant; * and ** indicate significant differences at the 0.05 and 0.01 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

Table 7

Mean values of several vegetative development parameters for each rootstock, irrigation method, and year, and the interaction (rootstock x irrigation method), for the period 2012–2016: NMS (number of main shoots per vine), LMS (length of the main shoot, m), AGR_{shoot} (absolute main shoot growth rate, $cm\ day^{-1}$, May–August period), NLS/MS (number of lateral shoots/main shoot), LLS (lateral shoot length, cm), TLA (total leaf area, $m^2\ vine^{-1}$), ELA (exposed leaf area, $m^2\ vine^{-1}$), pruning weight (PW, $kg\ vine^{-1}$), ELA/TLA ($m^2\ m^{-2}\ vine^{-1}$), and Toda index ($m^2\ kg^{-1}\ m^{-1}$).

	NMS	LMS	AGR_{shoot}	NLS/MS	LLS	TLA	ELA	PW	ELA/TLA	Toda index
Rootstock (R)										
140Ru	11.5bc	1.59b	1.36c	9c	7.29	4.92d	3.14d	0.89d	0.70a	0.66a
1103 P	11.0ab	1.51b	1.24c	10d	7.23	4.34c	3.00c	0.65c	0.76ab	1.04b
41B	11.9c	1.35a	1.12b	8bc	6.08	3.81b	2.69b	0.49b	0.80b	1.02b
110R	10.9a	1.28a	0.98a	6a	4.99	3.36a	2.54a	0.43a	0.90c	0.98b
161-49C	11.6c	1.30a	0.97a	8b	7.04	3.84b	2.68b	0.51b	0.80b	1.58c
Irrigation method (IM)										
PRI	11.3	1.42	1.13	8.1	6.45	4.10	2.83	0.60	0.80	1.12
RDI	11.5	1.39	1.14	8.5	6.59	4.01	2.79	0.59	0.79	0.99
Year										
2012	11.4a	1.53c	–	–	–	6.64d	3.35d	0.90e	0.52a	1.25c
2013	11.9b	2.04d	–	10b	4.19a	2.98b	3.16c	0.78d	1.04c	0.26a
2014	11.2a	0.76a	1.18b	5a	4.93a	2.52a	1.86a	0.24a	0.82b	1.88d
2015	11.1a	1.29b	1.09a	9c	10.45b	4.08c	2.87b	0.59c	0.78b	0.83b
2016	–	–	–	–	–	–	–	0.44b	–	–
Interaction (R x IM)										
140Ru PRI	11.2	1.58	1.29	9.2	8.56	4.89	3.15	0.84d	0.70	0.68
140Ru RDI	11.7	1.59	1.43	8.7	6.02	4.96	3.13	0.94e	0.69	0.64
1103 P PRI	11.3	1.51	1.29	9.7	5.78	4.44	3.03	0.67c	0.76	1.00
1103 P RDI	10.7	1.51	1.19	10.3	8.68	4.24	2.97	0.63c	0.76	1.08
41B PRI	11.8	1.34	1.13	7.7	6.45	3.80	2.67	0.49b	0.81	1.14
41B RDI	12.1	1.36	1.11	8.6	5.71	3.82	2.71	0.50b	0.80	0.90
110R PRI	10.7	1.34	1.04	6.3	5.07	3.66	2.65	0.48b	0.88	1.00
110R RDI	11.1	1.23	0.93	6.6	4.91	3.07	2.43	0.38a	0.91	0.95
161-49C PRI	11.5	1.34	0.92	7.4	6.42	3.73	2.62	0.51b	0.83	1.78
161-49C RDI	11.8	1.27	1.03	8.4	7.66	3.95	2.73	0.51b	0.77	1.38
ANOVA										
R	***	***	***	***	ns	***	***	***	***	***
IM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year	**	***	*	***	***	***	***	***	***	***
R x IM	ns	ns	ns	ns	ns	ns	ns	**	ns	ns

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

RGR of PRI berries was significantly lower than that of RDI berries (Table 6). Fresh weight accumulation rates during ripening (averaged for 2014 and 2015) and berry RGR post-veraison (in 2015) showed significant interactive effects of the rootstock and irrigation method (Table 6, Table 2S).

3.5. Vegetative development, yield response, sink-source relationships, and resources distribution

Vines grafted on rootstocks 140Ru and 1103P were the most invigorated plants, showing significantly greater trunk growth, absolute main shoot growth rate, final main shoot length, number of lateral shoots (1103P), total and exposed leaf area, and pruning weight than the vines grafted on the other rootstocks (Table 7, Table 3S, Supporting information). Rootstock 140Ru was also the most productive by far, with significantly higher % fructification, yield, number of clusters per shoot and per vine, cluster weight, berry weight, and berry number per cluster compared to the other rootstocks (Table 8 and 4S, Supporting information). This rootstock also induced greater cluster compactness and a higher % of big clusters (Table 5S, Supporting information). It was the most efficient from a productive point of view, with an averaged WUE_{yield} of $18\ kg\ m^{-3}$, compared to $9–11\ kg\ m^{-3}$ for the other rootstocks (Table 8). In addition, other whole-plant efficiency indices - such as $FW_{productivity}$, harvest index (HI), and DW_{yield} , - were highest for this rootstock (Fig. 8).

For vines grafted on 41B and 1103P, the vigor, productive response, and whole-plant WUE indices were intermediate. In contrast, vines grafted on rootstocks 110R and 161-49C were the least invigorated and productive (Tables 7 and 8), showing a significantly lower trunk growth

rate (Table 3S), WUE_{yield} , $FW_{productivity}$, $DW_{productivity}$, and DW_{yield} (Tables 8 and 9). The degree of cluster compactness and % of big clusters were also lower in these low vigor rootstocks, while their % of small clusters was the highest (in 2015, Table 5S). Also, vines grafted on 110R had the poorest vegetative development, the number of main shoots per vine, number of lateral shoots, total leaf area, exposed leaf area, and pruning weight being significantly ($P < 0.05$) lower than for vines grafted on 161-49C (Table 7). However, vines grafted on 161-49C were the least productive, the number of main shoots with clusters, % fructification, and number of clusters per shoot being significantly lower (Table 4S); consequently, they had lower yield and cluster weight than vines on 110R and the other rootstocks (Table 8). Besides, 161-49C was the rootstock with the lowest WUE from a productive point of view, although it did not differ significantly from 110R (Table 8).

There were no significant effects of the irrigation method (PRI or RDI) on any of the vegetative, productive, or WUE parameters measured. The vegetative development and yield were greatest in years 2012 and 2013 (rainy years) and poorest in 2014 (a warmer and drier year) (Table 8).

We observed a significant interaction between the rootstock and irrigation method for several parameters - such as pruning weight, yield, cluster weight, berry weight, and WUE_{yield} , (Tables 7 and 8). Thus, PRI vines grafted on 110R had significantly higher pruning weight, yield, and cluster weight than RDI 110R vines. In contrast, RDI vines grafted on 161-49C had significantly higher yield, cluster weight, berry weight, and WUE_{yield} than PRI vines (Table 8). Besides, RDI vines grafted on 140Ru had significantly higher pruning weight than PRI 140Ru vines.

Table 8

Average annual values of yield components at harvest for each rootstock, irrigation method, and year, and the interaction (rootstock x irrigation method) during the experimental period (2012–2016): WUE_{yield} (productive water use efficiency, kg m⁻³), Yield/PW (yield/pruning weight, kg kg⁻¹), TLA/yield (total leaf area/yield, m² kg⁻¹), and TLA/PW (total leaf area/pruning weight, m² kg⁻¹).

	Yield (kg vine-1)	Yield (kg ha-1)	Clusters vine-1	Cluster weight (g)	Berry weight (g)	Berries cluster-1	WUE _{yield}	Yield/PW	TLA/yield	TLA/ PW
Rootstock (R)										
140Ru	6.10d	16,258d	17c	349d	1.59c	225d	18.31c	7.85b	1.05a	5.89a
1103 P	3.69bc	9,836bc	15b	272c	1.39a	199c	11.07b	6.48a	1.61b	7.06ab
41B	3.91c	10,423c	13a	258bc	1.48b	177b	11.73b	8.97c	1.33a	8.10bc
110R	3.48ab	9,270ab	13a	250b	1.45bc	170ab	10.47ab	9.83c	1.15a	8.75c
161-49C	3.08a	8,227a	13a	217a	1.42a	158a	9.27a	7.24ab	1.77b	8.92c
Irrigation method (IM)										
PRI	4.06	10,768	14	269	1.46	186	12.13	7.79	1.39	7.74
RDI	4.05	10,791	14	269	1.47	185	12.20	8.36	1.37	7.75
Year										
2012	3.90bc	10,359bc	13b	292c	1.47c	197bc	11.88c	4.50a	1.80b	7.66b
2013	6.13d	16,344d	18d	331d	1.62e	204c	18.57d	8.06b	1.04a	3.94c
2014	2.34a	6,203a	10a	223a	1.41b	153a	7.45a	11.13c	1.59b	12.10a
2015	4.29c	11,441c	15c	269b	1.55d	187b	12.57c	7.86b	1.10a	7.28b
2016	3.60b	9,549b	15c	231a	1.28a	188bc	10.38b	8.82b	–	–
Interaction (R x IM)										
140Ru PRI	6.08d	16,222d	17	349d	1.62d	225	18.17d	7.92	1.02	5.99
140Ru RDI	6.11d	16,294d	17	348d	1.56cd	224	18.45d	7.78	1.07	5.80
1103 P PRI	3.73bc	9,949bc	14	274c	1.36a	206	11.15bc	6.26	1.68	6.78
1103 P RDI	3.65bc	9,723bc	13	270c	1.43ab	191	10.99bc	6.70	1.53	7.34
41B PRI	3.89c	10,372c	15	254bc	1.49bc	170	11.62c	8.91	1.37	8.51
41B RDI	3.93c	10,474c	15	262bc	1.46b	184	11.84c	9.02	1.30	7.70
110R PRI	3.82c	9,897c	14	268c	1.48bc	180	11.41bc	9.47	1.08	8.65
110R RDI	3.15ab	8,409ab	13	232ab	1.42ab	161	9.54ab	10.19	1.23	8.85
161-49C PRI	2.77a	7,399a	13	203a	1.36a	151	8.30a	6.37	1.81	8.77
161-49C RDI	3.40bc	9,055bc	14	232b	1.48bc	166	10.23bc	8.10	1.73	9.08
ANOVA										
R	***	***	***	***	***	***	***	***	***	***
IM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year	***	***	***	***	***	***	***	***	***	***
R x IM	*	*	ns	*	**	ns	*	ns	ns	ns

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

The sink/source indices calculated show that, in general, the most invigorating rootstocks (1103 P and 140Ru), particularly 140Ru, gave significantly lower ELA/TLA, TLA/pruning weight, Toda index, yield/pruning weight, and TLA/yield values than the rest of the rootstocks (Tables 7 and 8). Conversely, the low vigor rootstocks (110R and 161-49C) had, in general, the highest values of these ratios. In addition, of the invigorating rootstocks, 140Ru gave a significantly lower Toda index and TLA/yield ratio and a significantly higher yield/pruning weight ratio than 1103 P. Comparing the less invigorating rootstocks, 110R had significantly higher ELA/TLA and yield/pruning weight ratios and a significantly lower Toda index and TLA/yield ratio than 161-49C (Tables 7 and 8).

The differences in the sink/source ratios among rootstocks are supported by the data for the distribution of resources (water, fresh and dry matter) among plant organs. In general, invigorating rootstocks - such as 140Ru, followed by 1103 P and 41B - gave significantly greater accumulation of water and fresh and dry matter in seasonal plant organs than non-invigorating rootstocks (110R and 161-49C), although this depended on the year (Fig. 4). In particular, vines grafted on 140Ru accumulated significantly more water and fresh and dry matter in grapes than vines on other rootstocks. Conversely, 161-49C and 110R gave the lowest accumulation of water and fresh and dry matter in grapes. Also, the water content and dry and fresh matter in the main shoots were significantly lower for PRI vines, compared to RDI vines, in 2014 (a very dry year) (Fig. 5), but not in 2016 (data not shown). Interactions between the rootstock and irrigation method were also observed for the accumulation of water and fresh matter, particularly for the main shoots, clusters, and cumulative total (Fig. 5).

3.6. Berry composition and berry quality indices

Plants grafted on different rootstocks produced berries with different concentrations of TSS and titratable acidity, 161-49C giving the highest sugar concentration and tartaric/malic ratio, and the lowest pH and malic acid concentration (Table 9). The berries of the vines grafted on 161-49C and 140Ru had the highest titratable acidity values. In addition, the most vigorous rootstocks (1103 P and 140Ru) had the highest malic acid concentration (Table 9). Regardless of the rootstock, the irrigation method also had a significant effect on the pH, titratable acidity, IM (maturity index, °Brix/Titratable acidity), and tartaric/malic ratio, with PRI vines showing lower pH and IM and higher titratable acidity and tartaric/malic ratio than RDI vines (Table 9). A significant interaction between the rootstock and irrigation method was observed for malic acid, with vines grafted on 161-49C showing a significantly lower content with PRI than with RDI.

Vines grafted on rootstocks 140Ru and 41B produced the berries with the lowest color index of the must (Table 10). During the five years of study, an overall effect of the rootstock vigor on the concentration of phenolic compounds in the Monastrell grapes was observed. In general, higher concentrations of total anthocyanins, extractable anthocyanins, and polyphenols were produced by the less vigorous rootstocks (161-49C and 110R) (Table 10). The rootstock that gave the highest phenolic concentration throughout the experiment was 161-49C. The anthocyanins extractability was significantly greater (lower AE index) in 1103 P, compared to the other rootstocks. The seed maturity index was highest in vines grafted on 41B and lowest in vines grafted on 140Ru. A significant interaction (R x IM) was also observed for the color

Table 9

Technological maturity parameters of the must of Monastrell grapes at harvest, for five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C) and two different irrigation methods (PRI and RDI), from 2012 to 2016: TSS (total soluble solids, °Brix), titratable acidity (g tartaric acid L⁻¹), IM (maturity index, °Brix/Titratable acidity), tartaric acid (g L⁻¹), and malic acid (g L⁻¹).

		TSS	pH	Total acidity	IM	Tartaric acid	Malic acid	Tartaric/Malic
Rootstock (R)								
	140Ru	24.0ab	3.96b	3.63b	6.7a	4.51c	1.70c	3.04a
	1103 P	23.6a	4.07d	3.42a	7.0b	4.41bc	1.79c	2.93a
	41B	23.6a	3.95b	3.44a	7.0b	4.19a	1.50b	3.18a
	110R	24.3bc	4.01c	3.37a	7.3c	4.33ab	1.57b	3.16a
	161-49C	24.4c	3.91a	3.65b	6.8ab	4.37bc	1.39a	3.66b
Irrigation method (IM)								
	PRI	23.8	3.96	3.55	6.8	4.40	1.56	3.30
	RDI	24.1	4.00	3.45	7.1	4.32	1.61	3.09
Year								
	2012	25.5c	4.06d	3.33a	7.7c	4.63c	1.88d	2.54ab
	2013	21.4a	3.85b	3.67b	5.9a	3.77b	1.63c	2.38a
	2014	25.4c	4.15e	3.36a	7.6bc	5.23d	2.15e	2.56ab
	2015	22.4b	3.82a	3.75b	6.1a	4.55c	0.90a	5.66c
	2016	25.2c	4.01c	3.40a	7.5b	3.63a	1.38b	2.84b
Interaction (R x IM)								
140Ru	PRI	23.9	3.93	3.67	6.6	4.50	1.65cd	3.14
	RDI	24.0	3.99	3.60	6.8	4.51	1.75de	2.94
1103P	PRI	23.4	4.04	3.49	6.8	4.43	1.73de	3.06
	RDI	23.8	4.09	3.34	7.3	4.40	1.85e	2.81
41B	PRI	23.3	3.92	3.48	6.8	4.23	1.48b	3.27
	RDI	23.9	3.97	3.39	7.1	4.15	1.51bc	3.08
110R	PRI	24.2	4.00	3.42	7.2	4.32	1.64cd	3.04
	RDI	24.4	4.03	3.32	7.4	4.33	1.50bc	3.29
161-49C	PRI	24.4	3.89	3.69	6.8	4.52	1.32a	4.01
	RDI	24.3	3.93	3.61	6.8	4.23	1.46b	3.31
ANOVA								
	R	***	***	***	***	***	***	***
	IM	ns	***	**	**	ns	ns	*
	Year	***	***	***	***	***	***	***
	R x IM	ns	ns	ns	ns	ns	*	ns

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

intensity, total anthocyanins, polyphenols, and SM index (Table 10).

The antioxidant activity of the Monastrell grapes was determined during the period 2014–2016. There was a high, positive correlation between the antioxidant activity of the berry and its concentration of flavonoids ($r = 0.96$, $P < 0.001$, Fig. 4S, Supporting information). Grapes from vines grafted on 140Ru showed the lowest flavonoids concentration and antioxidant activity (Table 11). The rootstock also modified the concentrations of stilbenes in the berry - the concentrations of resveratrol, its glycosidic forms, and piceids being higher in a low productive-vigor rootstock such as 161-49C (Table 11). The rootstock also modified the concentration of total amino acids, which was highest in the berries of vines grafted on the rootstock 161-49C (Table 11). Vines receiving PRI produced grapes with higher flavonoids and stilbenes concentrations than those receiving RDI (Table 11). A R x IM interaction also occurred for total amino acids, with PRI vines grafted on 110R having a higher content than RDI vines and PRI vines grafted on 41B having a lower content than RDI vines (Table 11).

In order to have a more global quantitative view of the final berry quality, we calculated some quality indices (QIs) described by Romero et al. (2016c). According to these QIs, one of the less vigorous rootstocks, 161-49C, had the highest score for QI_{overall berry} (highest QI_{technological berry} and highest QI_{phenolic berry}), followed by 110R (Table 12). The lowest overall berry quality score was produced by the more vigorous rootstocks (140Ru and 1103P), due to a low QI_{technological berry} and QI_{phenolic berry}. In general, PRI vines had a significantly higher QI score than RDI vines, although a significant interaction between IS and R was also observed for the QI scores (Table 12). Thus, PRI vines grafted on 161-49C showed the highest QI score of all the combinations and PRI vines grafted on 140Ru had a higher QI_{overall}

berry score than RDI vines. In contrast, PRI vines grafted on 1103 P, 41B, or 110R had QI_{phenolic berry}, QI_{technological berry}, and QI_{overall berry} scores similar to those of RDI vines (Table 12).

3.7. Vine water status, berry quality, water use efficiency relationships

The phenolic quality (QI_{phenolic berry}), technological quality (QI_{technological berry}), and QI_{overall berry} showed significant curvilinear relationships with the averaged Ψ_s during the fruit set-veraison period, reaching their maximum values when Ψ_s was between -1.1 and -1.2 MPa (Fig. 6A–C). Conversely, QI_{phenolic berry} and QI_{overall berry} showed a significant linear relationship with the averaged Ψ_s during the veraison-harvest period (Fig. 6D and F), mainly during early ripening, with higher QI values occurring between -1.3 and -1.4 MPa (Figs. 6G–I). There was no significant relationship between QI_{technological berry} and the averaged Ψ_s maintained during the veraison-harvest period (Fig. 6E).

Berry quality (QI_{overall berry}, QI_{phenolic berry}) was linearly and negatively related with yield, WUE_{yield}, and fresh berry weight (Fig. 7A, D and H). Conversely, QI_{phenolic berry} and QI_{overall berry} were linearly and positively correlated with the TLA/yield ratio and exposed leaf area/TLA ratio, respectively (Fig. 7E and G). Besides, QI_{phenolic berry} increased with the Toda index (vine vigor index), reaching a certain saturation around $2 \text{ m}^2 \text{ kg}^{-1} \text{ m}^{-1}$ (Fig. 7F).

The FW_{productivity} aboveground and DW_{yield} were linearly and negatively correlated with berry QIs (Fig. 8 B, D, and F), but were positively correlated with the productive WUE_{yield} (kg FW m^{-3}) (Fig. 8A, C, and E). In addition, the HI was negatively correlated with QI_{phenolic berry} (Fig. 8G and H).

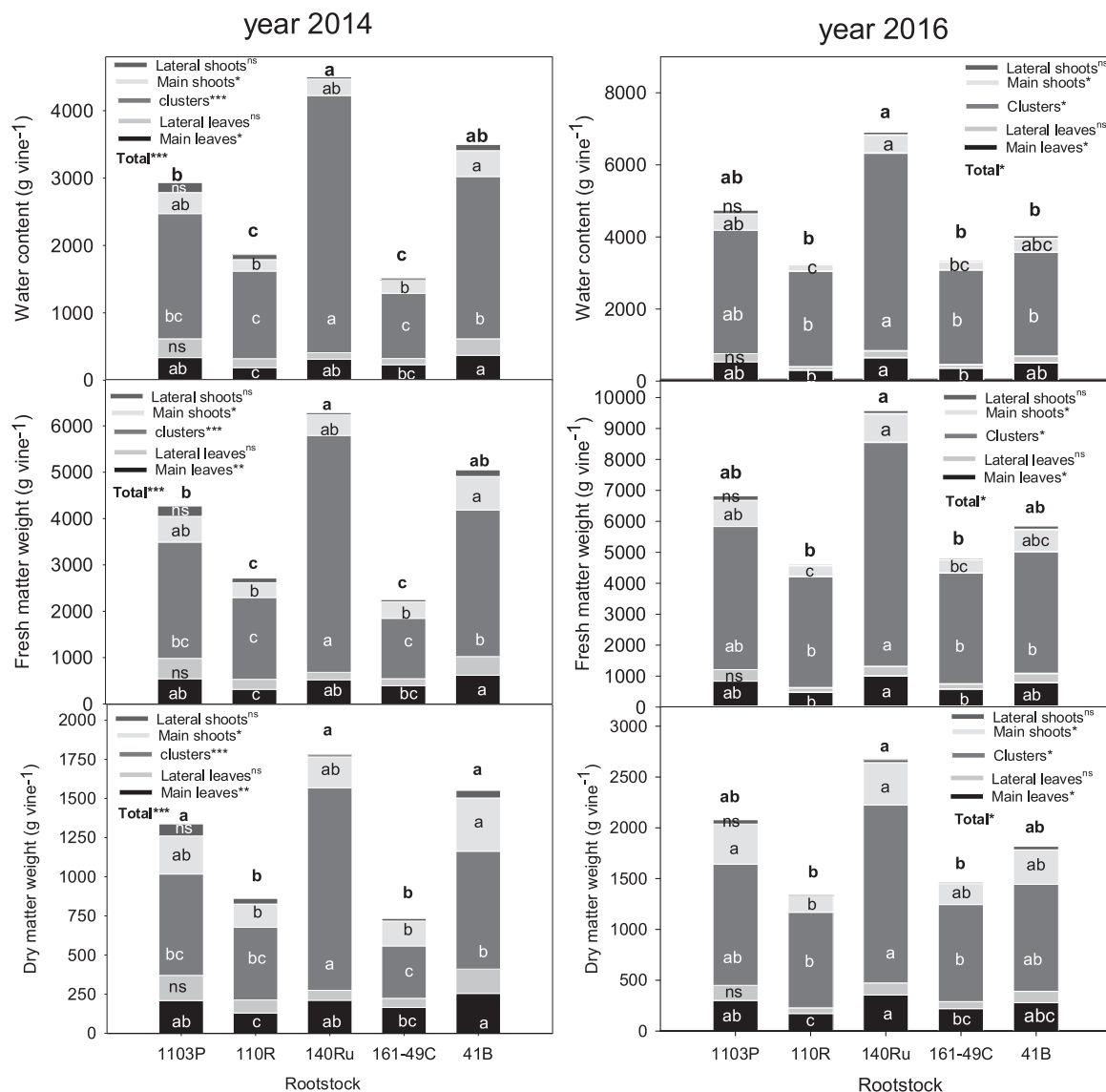


Fig. 4. Mean values of the (a) water content, (b) FW, and (c) DW of the main and lateral shoots, main and lateral leaves, and fruits in the grapevines for each rootstock, in 2014 and 2016. For each plant organ (main shoots, lateral shoots, leaves, and fruits), different letters within the different bars indicate significant differences among rootstocks. Different letters on the tops of the bars indicate significant differences in total plant values. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Separation was by Duncan's multiple range test at the 95% confidence level.

4. Discussion

4.1. Rootstock effect

The threat of climate change (CC) will make it necessary to combine different adaptation measures, especially those related to irrigation and water management and availability, in order to maintain the sustainability of vineyards (Iglesias and Garrote, 2015; Resco et al., 2016) - especially in the semiarid, warm, and more vulnerable winegrowing regions of Southern Europe (such as our study area). In this study, we combined the use of different rootstocks and DI with a low amount of water to increase WUE and improve vine performance and sustainability, as a long-term adaptation to CC. Our results show that the rootstock had a significant impact on the ability to extract water from the soil and, in consequence, on the vine water status, leaf photosynthesis, vigor, productivity, WUE, and berry quality of Monastrell vines. This mirrors previous studies with other rootstock-scion genotypes (Satisha et al., 2007; Peccoux, 2011; Marguerit et al., 2012; Berdeja et al., 2015; Zhang et al., 2016), but contrasts with other work

where no significant effect of the rootstock on vine performance was observed (Keller et al., 2012; Harbertson and Keller, 2012; Kidman et al., 2014). The studies examining rootstock effects on scion performance are highly variable in terms of results (Zhang et al., 2016). Differences in the edaphoclimatic and experimental conditions and specific rootstock-scion genotypic interactions may have contributed to these divergences (Keller et al., 2001; Santarosa et al., 2016).

Vines grafted on invigorating rootstocks - such as 1103 P or 140Ru, both classified as rootstocks with high or very high drought tolerance (Keller, 2010; Alsina et al., 2011; Lovisolo et al., 2016) - maintained greater water uptake capacity during the growing season and were able to exploit soil water resources more efficiently, increasing the irrigation efficiency. Improved root water uptake and transport capacity is likely due to the presence of *V. rupestris* in the genotypic background of 140Ru, 1103P, and 110R - compared to more drought susceptible rootstocks of lower vigor (*V. riparia* crossings), such as 41B or 161-49C (Lovisolo et al., 2016). This greater soil water uptake was also reflected in higher leaf nutrient content and vigor, superior accumulation of water and dry matter in plant organs and higher yield response. But this

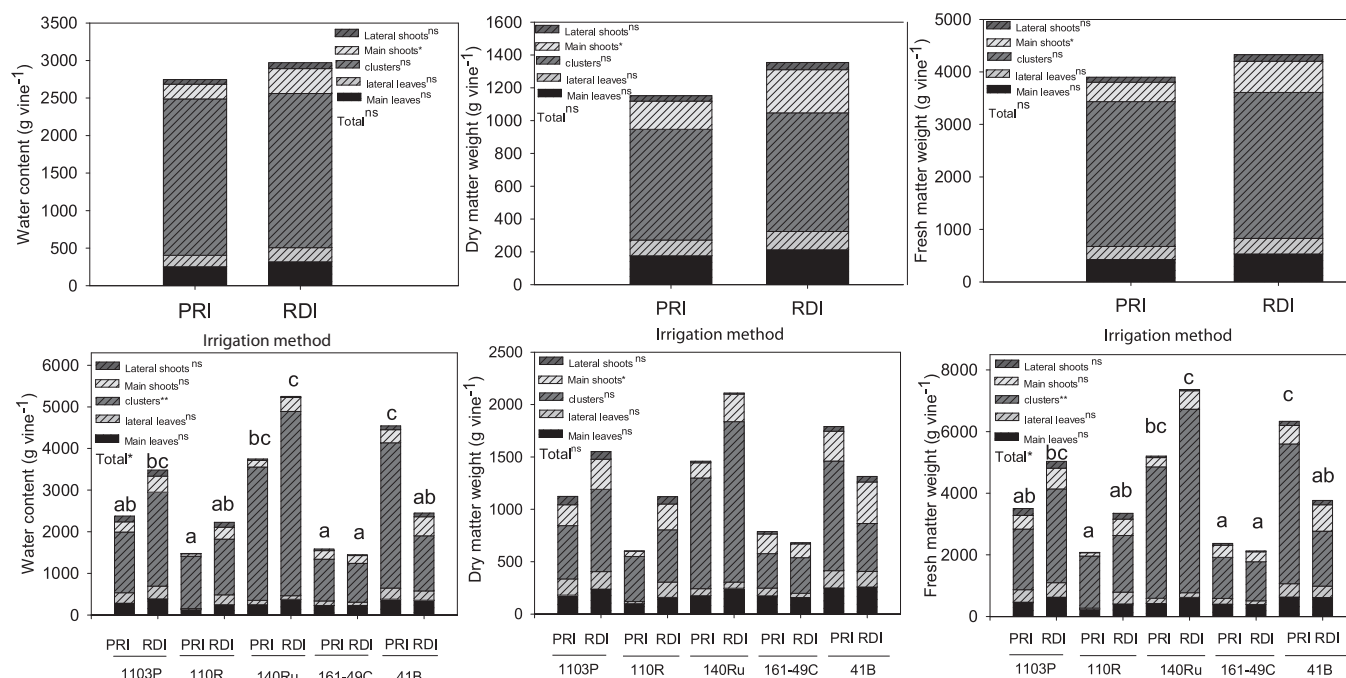


Fig. 5. Water and dry and fresh matter contents in different aboveground annual plant organs for different irrigation methods (PRI and RDI) and for the interaction (R x IM) at the end of the ripening period in 2014. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Separation was by Duncan's multiple range test at the 95% confidence level. Different letters on the tops of the bars indicate significant differences in total plant values.

Table 10

Phenolic maturity parameters of the must of Monastrell grapes at harvest, for five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C) and two different irrigation methods (PRI and RDI), from 2012 to 2016. Berry weight (g), TA (total anthocyanins, mg L^{-1}), EA (extractable anthocyanins, mg L^{-1}), polyphenols content (mg L^{-1}), AE index (anthocyanin extractability index, %), and SM index (seed maturity index, %).

		Color intensity	TA	EA	Polyphenols content	AE index	SM index
Rootstock (R)							
	140Ru	4.49a	482a	236a	34.9a	52.1b	73.5a
	1103P	5.02b	460a	236a	39.7b	48.2a	76.0bc
	41B	4.68a	491a	244a	43.0c	51.8b	77.3c
	110R	5.25b	552b	276b	43.8c	51.0b	74.7ab
	161-49C	5.06b	564b	287b	45.0c	52.1b	74.9ab
Irrigation method (IM)							
	PRI	4.77	518	261	41.6	50.6	74.9
	RDI	5.03	502	251	40.9	51.5	75.7
Year							
	2012	4.47b	734b	398d	46.2c	45.3a	64.7a
	2013	3.41a	355a	151a	33.9a	57.7c	82.3d
	2014	6.07c	531b	297c	48.2c	43.7a	75.0c
	2015	4.46b	358a	162a	42.8b	55.2b	85.4e
	2016	6.08c	573b	270b	35.3a	53.5b	69.1b
Interaction (R x IM)							
	140Ru						
	PRI	4.39a	494abc	243	37.3b	51.7	74.7bcd
	RDI	4.59ab	471ab	228	32.5a	52.5	72.4a
	1103 P						
	PRI	4.70abc	445a	234	40.0bc	46.4	76.0cd
	RDI	5.34de	476ab	239	39.5bc	50.1	75.9cd
	41B						
	PRI	4.43a	497bc	247	42.1cd	53.2	76.2cd
	RDI	4.93bcd	486ab	241	43.8de	50.5	78.5e
	110R						
	PRI	5.10cde	542c	276	42.4cd	50.4	74.0abc
	RDI	5.39e	563c	276	45.2de	51.6	75.5bcd
	161-49C						
	PRI	5.24de	613d	303	46.5e	51.5	73.4ab
	RDI	4.88bcd	516bc	270	43.6de	52.8	76.5de
ANOVA							
	R	***	***	***	***	**	***
	IM	**	ns	ns	ns	ns	ns
	Year	***	***	***	***	***	***
	R x IM	**	**	ns	**	ns	**

'ns' not significant; ** and *** indicate significant differences at the 0.01 and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

Table 11

Nutraceutical quality parameters of Monastrell grapes at harvest, for five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C) and two different irrigation methods (PRI and RDI), from 2014 to 2016. Antioxidant activity (TEAC index), flavonoids (mg catequin kg⁻¹ berries), piceid (mg kg⁻¹ berries), resveratrol-4'-O-glucoside (mg kg⁻¹ berries), resveratrol (mg kg⁻¹ berries), and total amino acids (mg leucine kg⁻¹ berries).

		Antioxidant activity	Flavonoids	Piceid	Resveratrol-4'-O-glucoside	Resveratrol	Total amino acids
Rootstock (R)							
140Ru		3.25a	946a	1.00a	0.87a	0.36a	841b
1103 P		3.69b	1,121b	1.24a	0.85a	0.35a	726a
41B		3.96b	1,204b	0.92a	0.89a	0.29a	631a
110R		3.89b	1,168b	1.05a	0.87a	0.28a	633a
161-49C		3.97b	1,181b	1.93b	1.78b	0.56b	940c
Irrigation method (IM)							
PRI		3.80	1,165	1.27	1.16	0.43	768
RDI		3.70	1,083	1.18	0.94	0.31	741
Year							
2014		1.37a	522a	0.56a	0.93a	0.19a	623a
2015		6.14c	1,697c	2.63b	1.38b	0.74b	574a
2016		3.74b	1,153b	0.48a	0.85a	0.18a	1066b
Interaction (R x IM)							
140Ru	PRI	3.44	1,013	1.14	1.14	0.48	828cde
	RDI	3.06	880	0.85	0.60	0.23	854de
1103 P	PRI	3.57	1,153	1.26	0.89	0.38	786bcd
	RDI	3.81	1,089	1.21	0.80	0.32	666ab
41B	PRI	3.98	1,271	0.94	0.94	0.31	544a
	RDI	3.93	1,138	0.89	0.85	0.27	718bc
110R	PRI	3.70	1,120	1.08	0.96	0.27	727bc
	RDI	4.08	1,217	1.01	0.78	0.29	539a
161-49C	PRI	4.31	1,269	1.94	1.89	0.71	953e
	RDI	3.63	1,092	1.91	1.68	0.42	927de
ANOVA							
R		**	***	***	***	***	***
IM		ns	*	ns	*	**	ns
Year		***	***	***	***	***	***
R x IM		ns	ns	ns	ns	ns	*

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

was offset by poorer final berry quality and color, lower polyphenolic and nutraceutical concentrations in the must, and lower QI scores, compared to rootstocks of medium-low vigor (41B, 110R, and 161-49C). This reflects previous results for other varieties (Nuzzo and Matthews, 2006; Nadal and Vernet, 2010; Wooldridge et al., 2010). It has been reported that, traditionally, high vine vigor and yields are associated with grapes and wines of low quality (Cortell et al., 2008).

Vines grafted on either of the invigorating rootstocks had sink-source ratios such as yield/pruning weight, TLA/yield, and the Toda index that were well outside the optimal agronomic ranges proposed for improving polyphenolic berry quality in DI Monastrell grapevines (Romero et al., 2016a), suggesting that these vines were unbalanced (Tables 7 and 8). Besides, their significantly lower ELA/TLA, compared to less vigorous rootstocks, probably indicates more closed canopies (higher proportion of shaded and internal leaves within the canopy) and decreased PAR in the cluster zone (data not shown), which can also negatively affect berry quality (Santos et al., 2007; Romero et al., 2010), in accordance with the linear and positive relationship between ELA/TLA and QI_{overall} berry (Fig. 7G).

Nevertheless, 1103 P and 140Ru, despite their genetic proximity (Sabir et al., 2010), and to irrigate with the same volume of water, differed in regulation of water relations and in their resources distribution, which had important repercussions for yield and berry quality. Thus, vines grafted on 140Ru maintained significantly better plant water status and leaf gas exchange and distributed more resources to berry growth and fruit load, but at the expense of the final grape quality and final QI scores (Tables 4,7,8, and 12). Conversely, vines grafted on 1103 P, had a significantly worse vine water status and leaf gas exchange and distributed more resources to leaf area development (lateral leaf area) and permanent structures (trunk growth) than to

berry growth and fruit load (Table 8) and gave better berry quality than 140Ru. Different factors - such as greater dilution as a consequence of larger berry size and higher fruit load (Romero et al., 2016b), greater cluster compactness, lower water stress during ripening, a lower degree of cluster homogeneity, and a higher % of big clusters (Table 5S, Supporting information) - may have contributed to reduce the final berry quality in 140Ru.

In general, rootstock 41B (classified as a rootstock with high, medium, low or very low drought tolerance, depending on the study, Peccoux, 2011) showed a moderate vigor-yield-berry quality response, clearly different from that of the other rootstocks, probably due to its different genomic constitution (it was the only rootstock derived solely from the crossing of *V. vinifera* (cv. Chasselas) x *V. berlandieri*) (Sabir et al., 2010).

Interestingly, Monastrell vines grafted on the least productive and least vigorous rootstocks - 161-49C (classified as having low-medium drought tolerance, Peccoux, 2011; Serra et al., 2013), followed by 110R (a rootstock of high-very high drought tolerance, Keller, 2010; Peccoux, 2011) - showed a significant improvement in long-term vine performance and berry quality under DI. This contrasts with other work where Syrah vines grafted on the rootstocks 110R and 161-49C showed poorer final berry quality than vines on more invigorating rootstocks (Novelli-Dias et al., 2017), revealing the importance of specific rootstock-scion interactions and experimental conditions.

Low vigor rootstocks (especially 161-49C) stimulated the accumulation of polyphenolics and improvements in technological parameters, giving the highest berry QI scores (Tables 9–12). Besides, they showed several other positive features, which may also have contributed to higher berry quality: lower cluster compactness, lower % of big clusters, higher % of small clusters and a higher degree of homogeneity in the

Table 12

Berry technological quality index (QI_{technological berry}), berry phenolic quality index (QI_{phenolic berry}), and overall berry quality index (QI_{overall berry}) calculated for Monastrell grapes at harvest, for five different rootstocks (140Ru, 1103 P, 41B, 110R, and 161-49C) and two different irrigation methods (PRI and RDI), from 2012 to 2016.

		QI _{technological berry}	QI _{phenolic berry}	QI _{overall berry}
Rootstock (R)				
140Ru		7.3a	2.5a	9.8a
1103 P		7.1a	2.9b	10.0a
41B		7.8b	3.0b	10.8b
110R		7.8b	3.4c	11.2b
161-49C		8.5c	3.8d	12.3c
Irrigation method (IM)				
PRI		7.9	3.2	11.2
RDI		7.5	3.0	10.5
Year				
2012		7.9c	4.7d	12.6d
2013		5.9a	1.9a	7.7a
2014		7.2b	3.4c	10.6b
2015		9.4d	2.3b	11.7c
2016		8.2c	3.3c	11.5c
Interaction (R x IM)				
140Ru	PRI	7.5bc	2.7ab	10.2bc
	RDI	7.1ab	2.3a	9.4a
1103 P	PRI	7.3abc	3.0bc	10.2bc
	RDI	6.8a	2.9bc	9.8ab
41B	PRI	7.9c	2.9bc	10.7cd
	RDI	7.8c	3.0bc	10.8cd
110R	PRI	7.8c	3.4c	11.2d
	RDI	7.9c	3.4c	11.3d
161-49C	PRI	9.1d	4.3d	13.5e
	RDI	7.9c	3.2bc	11.1d
ANOVA				
R		***	***	***
IM		**	*	***
Year		***	***	***
R x IM		*	*	***

'ns' not significant; *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

clusters (data not shown). However, the physiological and agronomic behaviors and plant resources distribution of 110R and 161-49C were clearly distinct. Thus, despite receiving the same volumes of water, vines grafted on 110R had a significantly higher root water uptake, better leaf nutrient (P)- plant water status and higher photosynthesis than vines grafted on 161-49C (Tables 3 and 4, Figs. 3 and 3S), confirming that 110R increased the water transport capacity, to maintain plant activity under water stress (probably also due to the presence of *V. rupestris* in its genotypic background, Lovisolo et al., 2016), and enhanced root aquaporins activity (Galmés et al., 2007) and other root traits (Peccoux, 2011; Barrios-Masias et al., 2015).

In accordance with the significant relationships found among the level of water stress, productive parameters, sink/source ratios, and berry quality indices (Figs. 6–8), the greater final berry quality in vines grafted on 161-49C, compared to 110R, could be due to several interacting factors. First, greater seasonal S_{ψ} and vine water stress during critical periods in 161-49C (Ψ_s values around -1.3 MPa were maintained during early ripening, within the optimum range proposed for Monastrell, (Romero et al., 2010), which could have stimulated the secondary metabolism in the berries (Castellarin et al., 2007). Second, greater leaf area development and carbohydrates supply - which could have had a positive effect, increasing the biosynthesis and accumulation of quality-related metabolites, both primary (sugars and acids) and secondary (anthocyanins). Third, lower berry weight, which could have enhanced the concentration of metabolites in the berries and QI_{phenolic}

berry (Fig. 7). Fourth, better balanced vines, with significantly lower yield/pruning weight ratios, a higher TLA/yield ratio (close to $1.8 \text{ m}^2 \text{ kg}^{-1}$) and Toda index ($> 1.5 \text{ m}^2 \text{ kg}^{-1} \text{ m}^{-1}$), and a high ELA/TLA ratio (open canopies and exposed clusters), as maintained in vines grafted on 161-49C (Tables 7 and 8), compared to R110, which stimulated more water and plant resources distribution to reproductive organs (higher yield than 161-49C, Table 8). All these features improved Monastrell berry quality (phenolic and technological) and nutraceutical potential under semiarid conditions and their values were close to or within the optimum physiological and agronomic thresholds and ranges proposed for Monastrell grapevines (Romero et al., 2016a).

The greater leaf Ca content in 161-49C vines (compared to the other rootstocks, Table 5) may indicate stronger adjustment to greater water stress intensity - as Ca is a player in most cellular signaling processes, acting as a secondary signal provoked by many different environmental stresses (Kacperska, 2004). Higher concentrations of total amino acids in berries from vines grafted on 161-49C were also probably a consequence of more severe water stress, since certain amino acids have functions - including antioxidant and osmoprotectant - that can protect abiotically-stressed plants (Cramer et al., 2013). Amino acids serve as precursors for the synthesis of wine volatile aromatic compounds, thus contributing to the overall taste of wine (Grimplet et al., 2009; Martins et al., 2012). In addition, stilbenes (resveratrol derivatives, piceids) are phytoalexins directly related with the intricate defense system used by plants against biotic and abiotic stresses (Jeandet, 2015). A higher concentration of stilbenes (as observed in 161-49C) can induce more protection against infection by plant pathogens (Signorelli and Guidoni, 2005). In this way, these low vigour vines (together with 110R vines) had lower affectation of fungal wood disease during the entire experiment (data not shown). An enhanced stilbenes concentration may have also important medicinal properties, leading to health benefits (Park et al., 2012).

4.2. Effects of the irrigation method (PRI vs RDI) and its interaction with the rootstock

Compared to RDI, PRI vines maintained a significantly lower root water uptake, a worse vine water status during ripening, and higher cumulative annual water stress (Tables 3 and 4, Figs. 2 and 3), confirming previous results in PRI Monastrell grapevines receiving low irrigation volumes (Romero et al., 2015).

Significant interactive effects also show that PRI vines decreased leaf N and P (161-49C, 140Ru), leaf Ca and Mg (1103 P), leaf Ca and Mn (41B), and leaf Zn contents (all rootstocks), compared to RDI vines (Table 5). These results suggest that the water applied in this study ($85\text{--}90 \text{ mm year}^{-1}$) and the volumetric soil water content maintained in the wet half of the root zone during most of the season were insufficient to maintain a more-favorable supply of water and certain minerals to the shoots in PRI vines, as observed in other edaphoclimatic conditions (Romero et al., 2016a, b) and suggest a decreased soil nutrient uptake/translocation capacity compared to RDI vines grafted on these rootstocks.

The altered soil-plant water relations and leaf nutrient status in PRI vines did not modify substantially the leaf photosynthesis, vigor, yield components, or productivity indices, compared to RDI vines (Tables 4–8). Conversely, berry quality parameters and nutraceutical potential were significantly improved by the PRI method, relative to RDI (Tables 9–12) in almost all rootstocks. Thus, in vines grafted on 140Ru, 1103 P, 110R and 161-49C the overall berry quality, phenolic-amino acid concentration and nutraceutical potential was significantly increased by PRI. In contrast, for PRI vines grafted on rootstock 41B, of medium vigor, there was no advantage in terms of yield, WUE, or berry quality (QI).

Probably, our results reflect intrinsic changes in vine physiology and berry metabolism caused specifically by PRI, as previously reported in Monastrell (Romero et al., 2016b) and in other grape varieties (Martin-

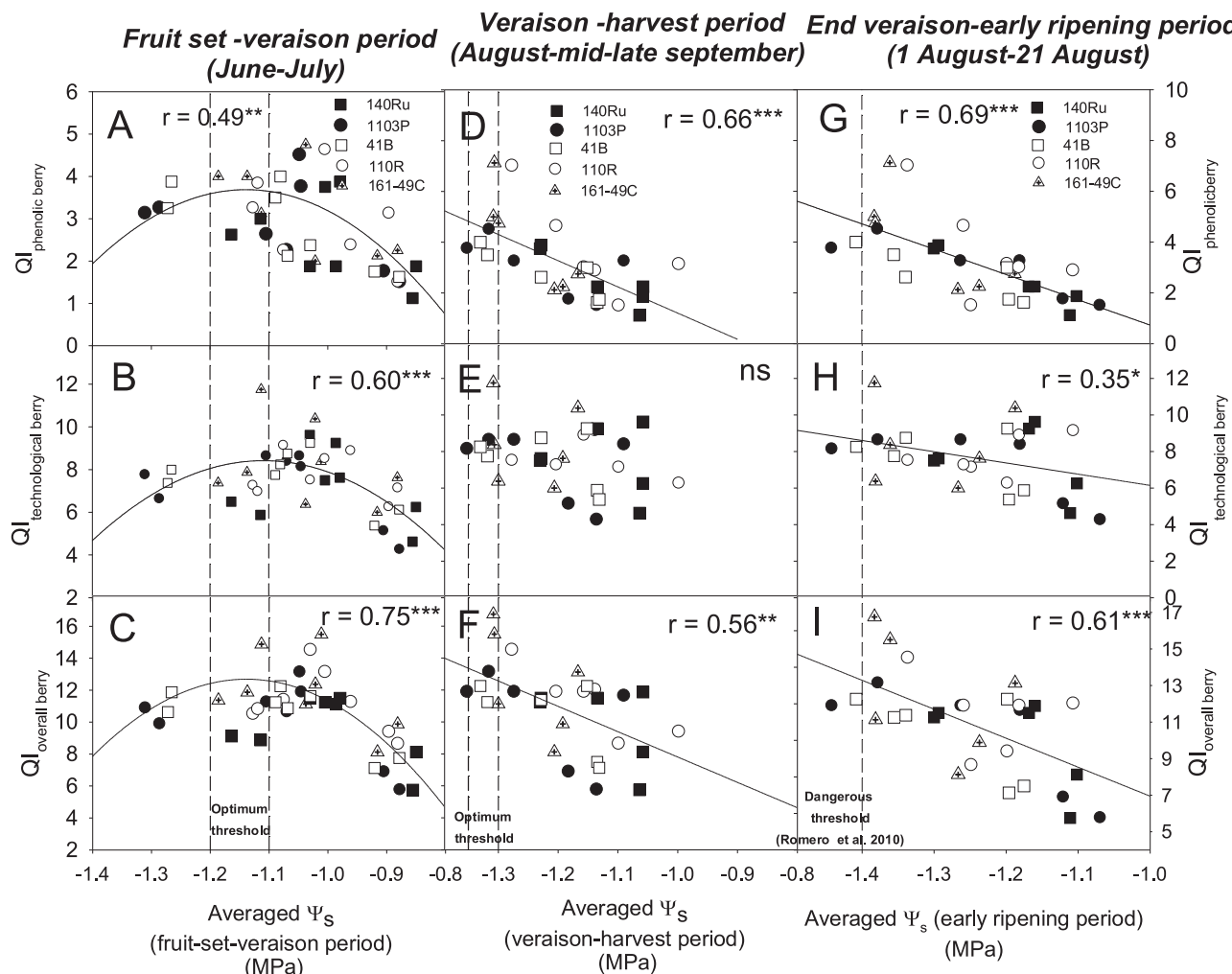


Fig. 6. Significant relationships between calculated berry quality indices (QI_{phenolic} , $QI_{\text{technological}}$, QI_{total}) and averaged midday stem water potential (Ψ_s) in different phenological periods: fruit set-veraison (A,B,C), veraison-harvest (D,E,F), and end of veraison-early ripening (G,H, I). ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Each point is the average of five years for the QI indices and four years for Ψ_s , calculated per plot for each rootstock. Vertical dashed lines represent optimum and dangerous thresholds of Ψ_s for, respectively, improving or damaging Monastrell berry quality.

Vertedor et al., 2004; Valdés et al., 2004, 2005; Uriarte-Hernández et al., 2005; Antolín et al., 2006, 2008; Bindon et al., 2008; Conesa et al., 2016). Previous research in PRI field-grown Monastrell vines showed an increased xylem sap ABA concentration following re-irrigation (Romero et al., 2014) and altered endogenous hormonal status of the berries (e.g. abscisic acid, ABA) at harvest, compared to RDI (Romero et al., 2015). Although we do not have berry ABA and other hormones concentration data in this study, ABA is implicated in berry ripening process and grapevine response to stress (Ferrandino and Lovisolo, 2014), and berry ABA accumulation can induce a selective increases in the transcript abundance of transcription factors and key genes of the flavonoids biosynthesis, encoding catalytic enzymes of the phenylpropanoid pathway (Villalobos-González et al., 2016), which may explain the increase in the concentration of flavonoids and stilbenes in PRI. In addition, in winegrapes, the intrinsic characteristics of the soil (fine textured soil as in this study: clay-loam or clayey soils as in previous Romero et al., studies), also promotes the release of non hydraulic signals (root-to shoot hormonal signalling, putatively ABA) (Tramontini et al., 2013), which probably favours the application of PRI method, and an improvement in the final berry quality. In other soil types (e.g. sandy soils) which do not promote root-to-shoot stress signalling (Tramontini et al., 2013), PRI effects on berry quality may not be beneficial.

Interestingly, the effects of PRI differed between the two low vigor

rootstocks, 110R and 161-49C. Thus, PRI vines grafted on 110R showed enhanced pruning weight, yield (+1,400 kg ha⁻¹, Table 8), total amino acids, and resveratrol and a similar QI score compared to RDI vines grafted on 110R. The higher yield and berry weight of PRI vines, with a similar polyphenolic concentration and must quality and greater nutraceutical potential, suggests no dilution, but, instead, greater synthesis and accumulation of photoassimilates and secondary metabolites in the berries relative to RDI vines (De la Hera et al., 2007). In contrast, 161-49C vines under PRI maintained similar vigor and lower berry weight, yield (−1,700 kg ha⁻¹), and WUE_{yield}, compared to RDI vines, but had the highest $QI_{\text{technological}}$ berry, QI_{phenolic} berry, and QI_{overall} berry scores and nutraceutical potential of all the combinations. The coincidence of higher phenolic concentration and QI scores with lower berry weight and yield for PRI (as consequence of greater water stress) also suggests a significant concentration effect for secondary metabolites in the berries, compared to RDI (Romero et al., 2016b).

Despite the higher berry quality, an excessive yield penalty in 161-49C PRI vines indicates greater long-term cumulative water stress effects and can make PRI unviable in this rootstock overall (relative to RDI), taking into account the higher installation costs of PRI (García-García et al., 2012). This rootstock-scion-irrigation method combination, under DI with low water volumes, would give low yields of berries of high quality and nutraceutical potential for the production of premium red wine with a high added value. Unfortunately, the current

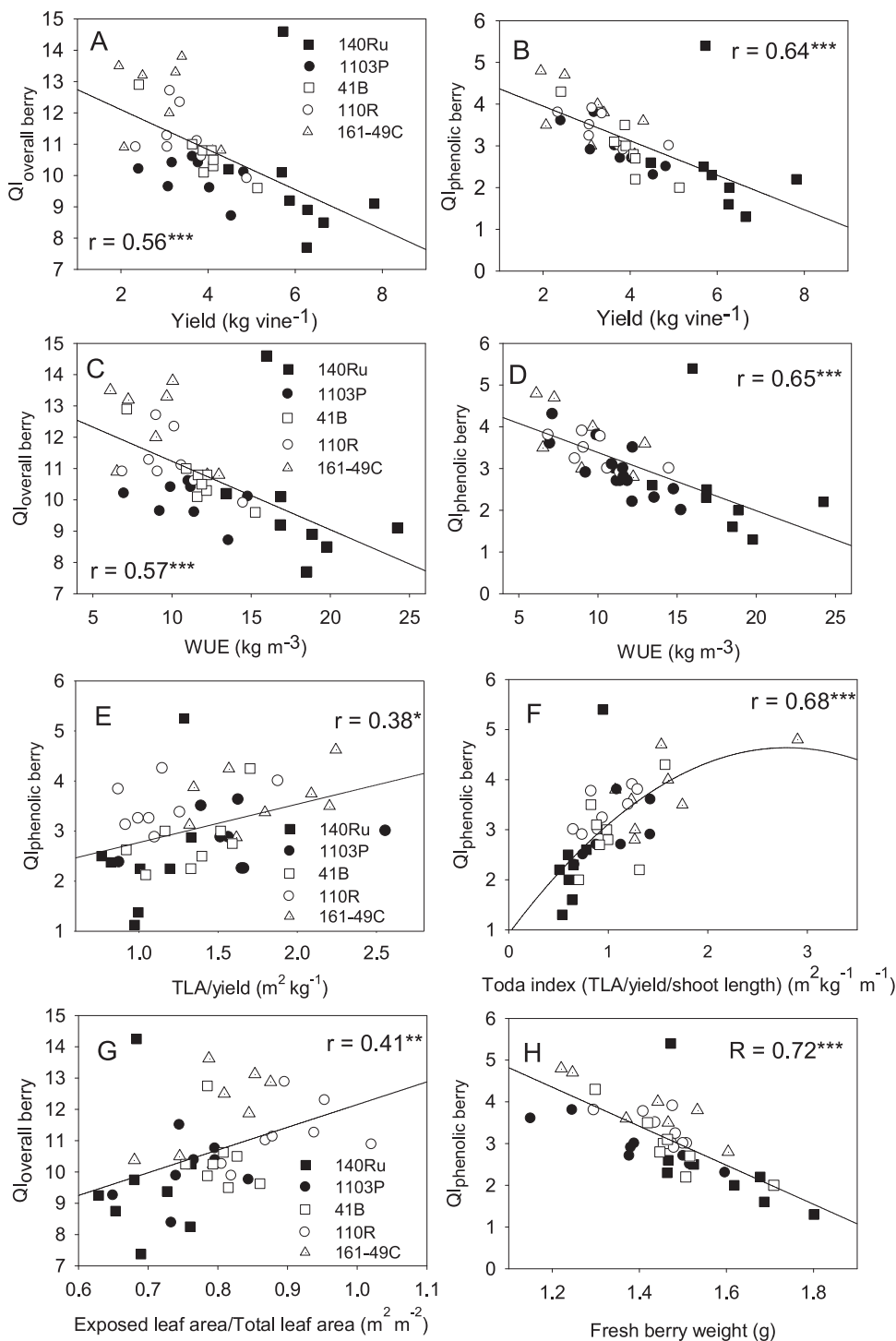


Fig. 7. Significant relationships between yield and $QI_{\text{overall berry}}$ (A) and between yield and $QI_{\text{phenolic berry}}$ (B). Significant relationships between WUE and $QI_{\text{overall berry}}$ (C) and between WUE and $QI_{\text{phenolic berry}}$ (D). Significant relationships between TLA/yield ratio and $QI_{\text{phenolic berry}}$ (E) and between Toda index and $QI_{\text{phenolic berry}}$ (F). Significant relationships between exposed leaf area/total leaf area ratio and $QI_{\text{overall berry}}$ (G) and between fresh berry weight and $QI_{\text{phenolic berry}}$ (H). ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. For A–D and H, each point is the average of five years, calculated per plot for each rootstock. For E–G, each point is the average of four years, calculated per plot for each rootstock (2012–2015).

grape market conditions, where the payment system in many areas still uses kg oBaume, without taking into account other quality parameters (such as the phenolic concentration and nutraceutical potential), favor high productivity – to the detriment of better grape quality and vineyard efficiency (Romero et al., 2016b). Other combinations – such as vines grafted on 110R under PRI (9,900 kg ha^{-1}) and vines grafted on 161-49C under RDI (9,055 kg ha^{-1}) – also gave high grape quality and similar $QI_{\text{overall berry}}$ scores (> 11 , Table 12) and can be good options to seek a compromise between long-term yield-quality-efficiency and returns for the grower.

4.3. Implications for vineyard irrigation management in semiarid conditions

In this winegrowing area, a DI strategy with low annual water volumes (85–90 mm year^{-1}) was enough to maintain optimum physiological vine performance during the season, give moderate or high yields (7,000–16,000 kg ha^{-1} , depending on the rootstock) and high WUE_{yield} levels (9–18 kg m^{-3}) and berry quality for premium red wine production. The ranges of yield (7,400–9,900 kg ha^{-1}) obtained with low vigour rootstocks 110R and 161-49C are adequate and fit well the

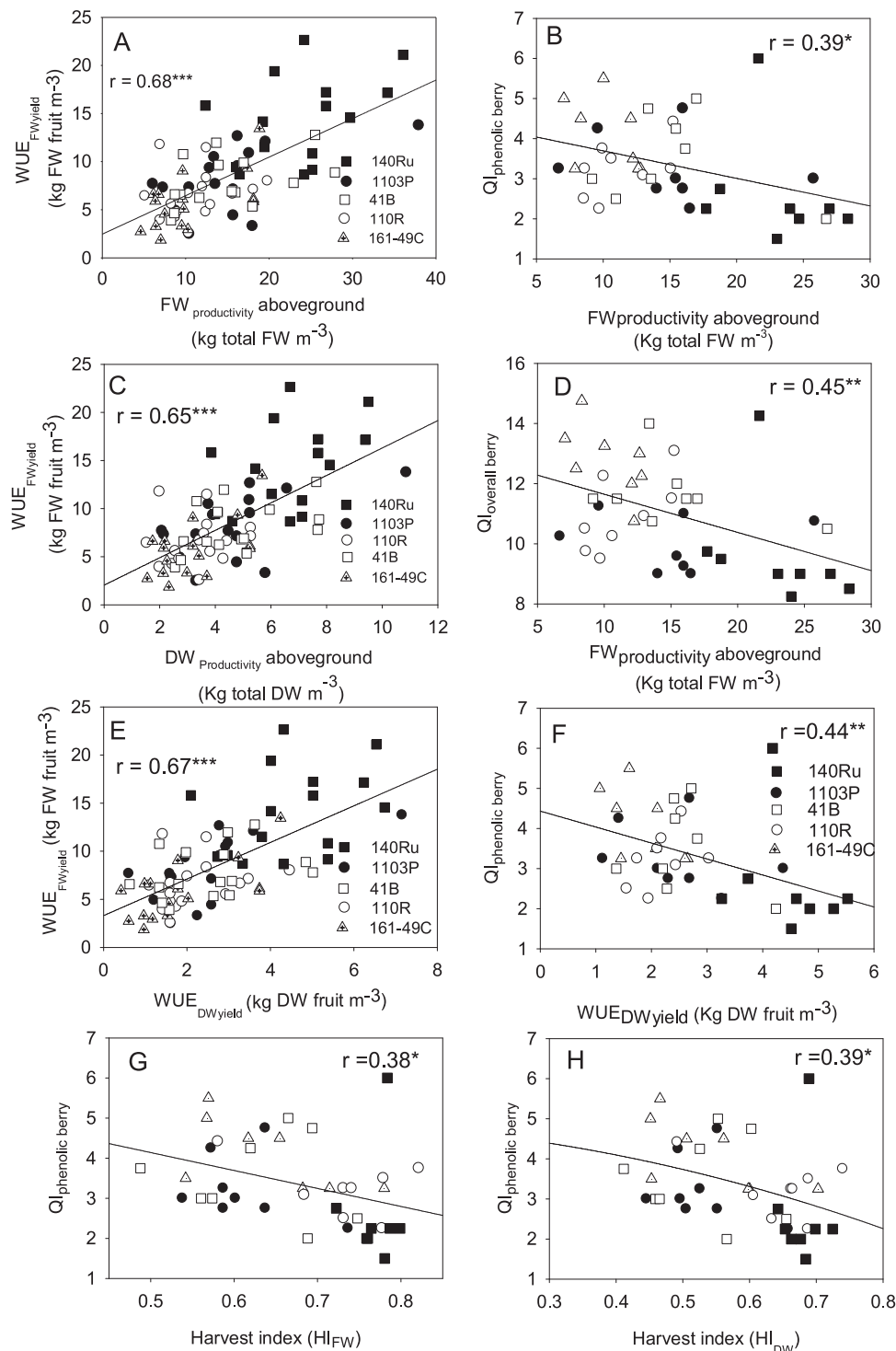


Fig. 8. Significant relationships between FW_{productivity} aboveground and WUE_{FWyield} (A) and QI_{phenolic berry} (B). Significant relationships between DW_{productivity} aboveground and WUE_{FWyield} (C) and between FW_{productivity} aboveground and QI_{overall berry} (D). Significant relationships between WUE_{DWyield} and WUE_{FWyield} (E), between WUE_{DWyield} and QI_{phenolic berry} (F), and between Harvest index (expressed as FW, HI_{FW} (G) or DW, HI_{DW} (H)) and QI_{phenolic berry}. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Each point is the average of two years (2014 and 2016), calculated per plot for each rootstock.

upper limit established by D.O. Bullas for red wine production, while the yield of 16,000 kg ha⁻¹ obtained with invigorating rootstock 140Ru surpasses these limits by far. In addition, the Θ_v in deeper soil layers (60–100 cm) changed little during most of the growing season (Fig. 1S), indicating minor deep percolation, according to the low irrigation volumes applied.

The DI strategy applied in this study with 1) no stress or slight stress from budburst to fruit set (19–20 mm year⁻¹), moderate-severe water

stress from fruit set to harvest (23–24 mm year⁻¹), partial irrigation recovery after veraison (35–36 mm year⁻¹) to maintain moderate stress from veraison to harvest and full recovery postharvest (9 mm year⁻¹) (Table 2), it was in general, adequate to maintain the vine water status within an optimum range of moderate water stress ($-1.2 \geq \Psi_s > -1.40$ MPa) during critical periods of the growing season, never reaching dangerous values (< -1.4 MPa) in any rootstock (Fig. 3S). The significant QI- Ψ_s relationships indicate that the maintenance of Ψ_s

between -1.1 and -1.2 MPa from fruit set to veraison and between -1.3 and -1.4 MPa from veraison to harvest maximizes berry QI in Monastrell grapevines (Fig. 6), agreeing with Romero et al. (2010) and these ranges can be useful for the management of physiologically-based irrigation techniques.

The water volumes applied in this study are well below the recommended thresholds for Monastrell grapevines under semiarid conditions in other wine growing region of SE Spain, D.O. Jumilla (≥ 110 mm year $^{-1}$, the optimum irrigation being 140–150 mm year $^{-1}$) (Romero et al., 2013, 2016b). Probably, the different rootstocks used and distinct edaphoclimatic conditions (60-cm-deep fine clay soil with 48% clay, and a hotter and drier area with 276 mm rainfall year $^{-1}$, in the Jumilla study, vs. 80-cm-deep clay-loam soil with 33% clay in a less warm and slightly rainier area (302 mm rainfall year $^{-1}$), in this Bullas study) can help explain these differences (Romero et al., 2016a, b).

At veraison, there were no important leaf deficiencies of macro and micronutrients, although the values are close to the lower limits of the optimum ranges proposed for whole grape leaves in irrigated vineyards (Davenport and Horneck, 2011). This indicates that a long-term, low nutrient input strategy, as applied in our edaphoclimatic conditions, can be adequate for Monastrell grapevines under DI.

Previous research also indicated that the benefits of PRI - relative to DI - in different species depend on the soil water content maintained in the wet root zone and the total soil water availability (Hu et al., 2011; Hutton and Loveys, 2011; Wang et al., 2012; Einhorn et al., 2012; Beis and Patakas, 2015; Romero et al., 2016a, b), which are determined by the irrigation management (frequencies and volumes). However, the results of the present study indicate that, depending on the specific rootstock-scion interaction, PRI can also be beneficial for Monastrell yield or berry quality, even with low irrigation water volumes, decreased θ_v availability in the wet root zone and lower root water uptake compared to RDI. Thus, compared to RDI, the application of PRI with low water volumes improved substantially Monastrell yield response (110R) and berry quality and nutraceutical potential - especially for low vigor rootstocks (161-49C, 110R) and two invigorating rootstocks (140Ru, 1103P). In addition, compared to RDI method, PRI method showed deeper water percolation.

Although currently vineyards in South Eastern Spain (such as Bullas D.O.) are mostly maintained without irrigation - with rainfall 300 mm year $^{-1}$ and high summer temperatures, and frequent drought waves - the threat of CC in these semiarid zones, with a probable warmer and drier climate, will make them unfeasible in the near future. In this situation, the application of low irrigation volumes (85–90 mm year $^{-1}$), in combination with low fertilizer inputs, low vigor rootstocks (161-49C and 110R), and well-designed DI strategies (PRI and RDI) it could be an adaptation to CC in order to guarantee the sustainability of the Monastrell vineyards under semiarid conditions. Besides, for the invigorating, drought tolerant rootstocks 140Ru and 1103P, our results show that irrigation could be reduced even more under optimized DI strategies in order to control the excess vigor and yield. In subsequent studies, we will seek to confirm or undermine this hypothesis and determine whether these physiological changes and altered berry quality, conditioned by the rootstock and irrigation method, are reflected in wine composition and wine sensory attributes.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agwat.2018.07.012>.

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